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Biochemical and Biophysical Research Communications 323 (2004) 49–51
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Independence of hyperleptinemia-induced fat disappearance from thyroid hormone☆

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Received 19 July 2004
Available online 28 August 2004

Abstract

Sustained hyperleptinemia induced in normal rats causes the rapid disappearance of body fat. This is attributed to a marked increase in uncoupled fatty acid oxidation in the white adipocytes, which also occurs in hyperthyroidism. Because hyperleptinemic rats have normal plasma T3 or T4 levels, we tested the possibility of "localized hyperthyroidism" due to increased conversion of T4 to T3 in the adipose tissue. We therefore induced sustained hyperleptinemia in normal rats by intravenous injection of recombinant adenovirus containing the leptin cDNA (AdCMV-leptin) and measured the mRNA and the activity of enzymes involved in T4 metabolism in the disappearing fat. The epididymal fat pad remnants exhibited a decrease in mRNA of deiodinase 1 and a doubling of deiodinase 2 mRNA ($p < 0.05$), but their enzyme activities did not differ from normoleptinemic controls. To determine if thyroid hormone was required for the fat-wasting action of hyperleptinemia, we infused AdCMV-leptin into rats made athyroid by total thyroidectomy or by methimazole therapy. The fat loss in hyperleptinemic athyroid rats was as great as in euthyroid controls. We conclude that the fat-wasting effect of sustained hyperleptinemia does not involve "local hyperthyroidism" in white adipose tissue and does not require thyroid hormone.

Sustained hyperleptinemia causes normal rats to lose all visible body fat with 7–14 days [1–3]. The lipid depletion involves a massive increase in uncoupled fatty acid (FA) oxidation inside the white adipocytes. This is attributed to the dramatic increase in their mitochondria [3], probably stimulated by the striking rise in peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) [4]. The energy generated by the FA oxidation is presumably dissipated as heat, given the increased expression of uncoupling protein (UCP)-1 and -2 mRNA measured in the disappearing fat [3,5]. Neither PGC-1α, UCP-1 nor -2 is normally expressed in white fat, but both PGC-1α and UCP-2 can be induced by thyroid hormone [6,7]. Indeed, the increase in uncoupled FA oxidation induced by hyperleptinemia probably occurs in only two other conditions, hyperthyroidism [7] and the extraordinarily rare Luft syndrome [8]. Since plasma T4 and T3 levels were normal in hyperleptinemic rats [3], the report that intracerebroventricular administration of leptin prevents the normal decrease in the conversion of T4 to T3 induced by fasting [9] raised the possibility that the leptin-induced conversion of white adipocytes from fat-storing to fat-burning cells was due to a local increase in conversion of T4 to T3 in white adipocytes. To test this possibility we again induced

☆ *Abbreviations:* DIO1 and 2, deiodinase types 1 and 2; ZDF, Zucker diabetic fatty.

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doi:10.1016/j.bbrc.2004.08.057

intense hyperleptinemia by administering intravenously recombinant adenovirus containing the leptin cDNA and examined the expression levels and enzyme activities of 5′-monodeiodinase types 1 and 2 (DIO1 and 2) in the disappearing adipose tissue. We also compared the effect of hyperleptinemia on fat disappearance in euthyroid and athyroid rats.

Materials and methods

Animals. Locally bred, 6-week-old lean, male wild-type Zucker diabetic fatty (ZDF) rats (+/+) were housed in individual cages. They were allowed ad libitum access to water and food. Food intake and body weight were measured regularly.

Inducing thyroid deficiency. Athyroidism was induced either by total thyroidectomy or by adding 0.04% of methimazole (2-mercapto-1-methyl-imidazole, Sigma) to the drinking water. Controls for thyroidectomized group were sham-operated rats, while controls for methimazole-treated rats received no treatment. Plasma specimens were obtained 20 days later and assayed for T3 and T4 by radioimmunoassay using the DiaSorin, Stillwater, MN kit.

Inducing hyperleptinemia. Recombinant adenovirus containing either the rat leptin cDNA (AdCMV-leptin) or β-galactosidase cDNA (AdCMV-β-Gal) as a control was prepared and administered intravenously 21 days after thyroidectomy or methimazole treatment, as previously described [1]. Body weight and food intake were measured daily.

Quantitative real-time PCR. mRNA from epididymal fat pad from β-Gal-treated rats or leptin-treated rats was extracted using TRIzol reagent (Invitrogen, Life Technologies, CA). First strand cDNA was synthesized from 2 μg of DNaseI-treated total RNA (DNase-free, Ambion) with random hexamer primers using the reverse transcription reagents kit (Applied Biosystem, Foster City, CA). Specific primers for each gene (Table 1) were designed using Primer Express software. The real-time PCR contained, in a final volume of 10 μl, 10 ng of reverse transcribed total RNA, 167 nM of the forward and reverse primers, and 5 μl of 2× SYBR green buffer (Applied Biosystem, Roche Molecular Systems, NJ). PCRs were carried out in triplicate in 384-well plates, using the ABI PRISM 7900HT Sequence Detection System. The relative amount of mRNAs was calculated using the relative standard curve method (bulletin No. 2, ABI PRISM 7700 sequence detection system). Ribosomal 18S mRNA was used as the invariant control.

Deiodinase activity assays. Tissue was homogenized in 10 volumes of 0.1 M phosphate (pH 7.2), 2 mM EDTA, and 10 mM DTT (P100E2D10 buffer). D2 activity was assayed in fresh homogenates, and the remainder was aliquoted, snap-frozen, and stored at −80 °C until analysis of D1 and D3 activities. Deiodinase activities were assayed by monitoring the preferred reaction catalyzed by the different isoenzymes, i.e., ORD of rT3 by D1 and ORD of T4 by D2. D1 and D2 activities were assayed by measurement of the release of radioiodide from outer ring labeled substrates [10,11].

D1 activity assay. Epididymal fat homogenates were incubated for 30 min at 37 °C with 0.1 μM rT3 and 10^5 cpm [3′,5′-^{125}I]rT3 in 0.1 ml P100E2D10 buffer. Blank incubations were carried out in the absence of homogenate. The reactions were stopped by adding 0.1 ml ice-cold 5% BSA in water, and radioactive iodothyronines were precipitated by adding 0.5 ml ice-cold 10% (wt/vol) trichloroacetic acid in water. After centrifugation, radioiodide was further isolated from the supernatants on Sephadex LH-20 mini-columns as previously described [11]. Deiodinase activity of homogenates was corrected for nonenzymatic deiodination observed in the blanks.

D2 activity assay. Epididymal fat pad homogenates were incubated for 60 min at 37 °C with 1 nM (10^5 cpm) [3′,5′-^{125}I]T4 in the presence of 100 nM T3 to block any D3 activity, in the presence of 0.1 mM PTU to block any D1 activity, and in the absence or presence of 100 nM unlabeled T4 to saturate D2 in 0.1 ml P100E2D10 buffer. Blank incubations were carried out in the absence of homogenate. Release of $^{125}I^-$ was determined and corrected for nonenzymatic deiodination as described above.

Table 1
Sequences and accession numbers for primers (forward, FOR and reverse, REV) used in real-time RT-PCR

Gene	Sequence for primers (FOR and REV)	Accession No.
18S	From ribosomal RNA control reagents kit (PE Applied Biosystems)	
DIO1	FOR: CGATTCGCCCCTGACAACT REV: ACCTTCAGGACGAACCAGAAGTAC	NM_021653
DIO2	FOR: GCGCTCTATGACTCGGTCATT REV: TCCGCGAGTGGACTTGGA	NM_031720

Results and discussion

DIO1 and DIO2 mRNAs were measured in epididymal fat pads of rats treated with either β-Gal or leptin adenovirus for seven days. As expected, body weight and food intake decreased in the hyperleptinemic rats as their fat disappeared. There was no difference in DIO1 mRNA between AdCMV-leptin-treated rats and the AdCMV-β-Gal-treated controls but DIO2 mRNA was significantly increased ($p < 0.05$) (Fig. 1). However, there was no difference in enzyme activity of either DIO1 or DIO2 enzymes (data not shown).

To determine if the disappearance of fat induced by the hyperleptinemia required the presence of thyroid hormone, we induced hypothyroidism in 6-week-old (+/+) ZDF rats, either by complete removal of the thyroid gland or by treatment with 0.04% methimazole in drinking water. After 21 days T4 and T3 were undetectable in all rats. Nevertheless, the response of the athyroid rats to AdCMV-leptin-induced hyperleptinemia was as great as that of euthyroid rats (Fig. 2). The reduction in food intake and body weight did not differ and, on postmortem examination, no white fat was visible in either group.

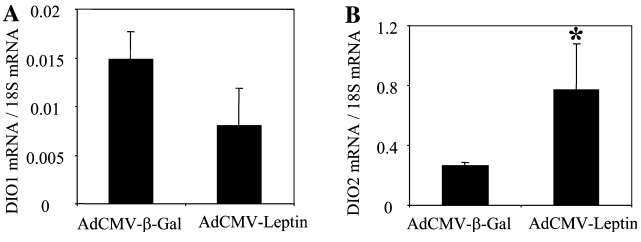

Fig. 1. Comparison by real-time RT-PCR of DIO1 mRNA/18S mRNA (A) and DIO2 mRNA/18S mRNA (B) in ZDF (+/+) rats treated with AdCMV-β-Gal or AdCMV-leptin for seven days ($n = 4$ in each group, $^*p < 0.05$).

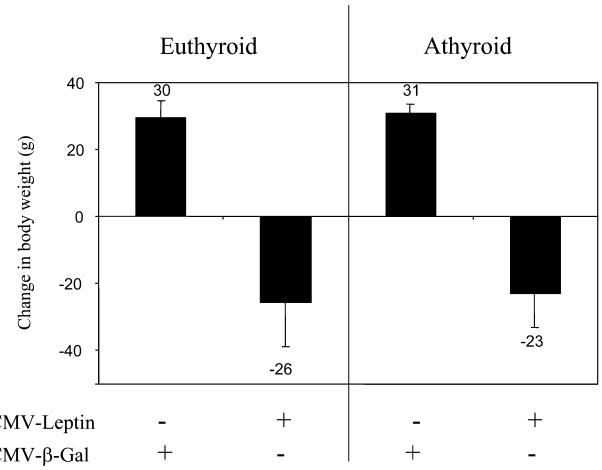

Fig. 2. Comparison of the effect hyperleptinemia on body weight of euthyroid and athyroid rats eight days after injection of either AdCMV-leptin or AdCMV-β-Gal.

We conclude from these results that the fat-disappearing action of sustained hyperleptinemia in normal rats is not the result of localized hyperthyroidism in adipocytes mediated by leptin-induced increase in conversion of T4 to T3 through upregulated DIO2. In fact, the full effect of hyperleptinemia on fat disappearance occurs in the absence of measurable thyroid hormone.

Acknowledgments

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-02700 and Merit Review No. 821-103. We thank Dr. Caroline Aspord for assistance with thyroidectomy and Christie Fisher for secretarial support. We thank Drs. Leslie Orci and Françoise Rohner-Jeanrenaud for useful discussions.

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