Ascertainment of changes in thyroid hormones metabolism in white adipose tissue by radiometric enzyme assays

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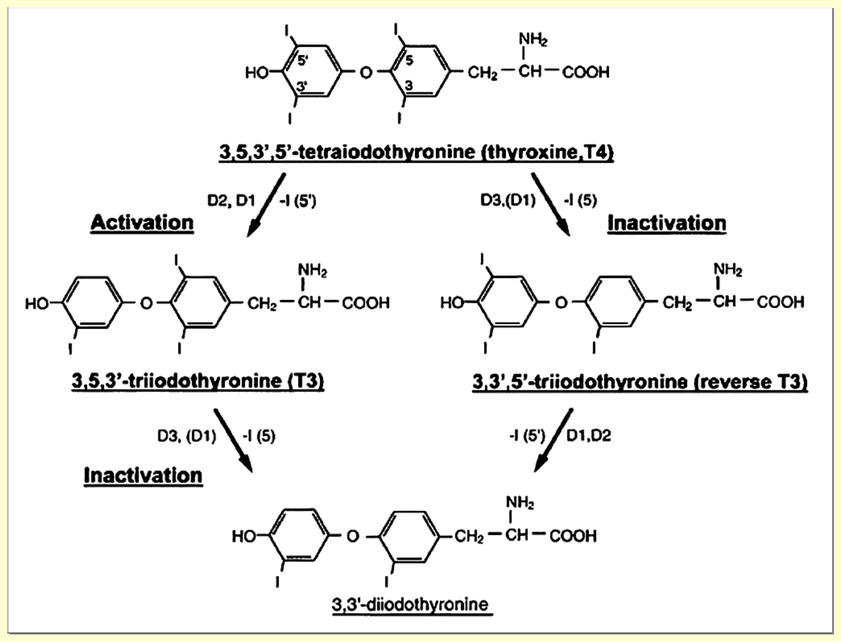
INTRODUCTION

Thyroid hormones (TH) play important roles in the development and function of both, brown (BAT) and white adipose tissue (WAT). However, data about local transformations of TH in WAT are still scarce [1].

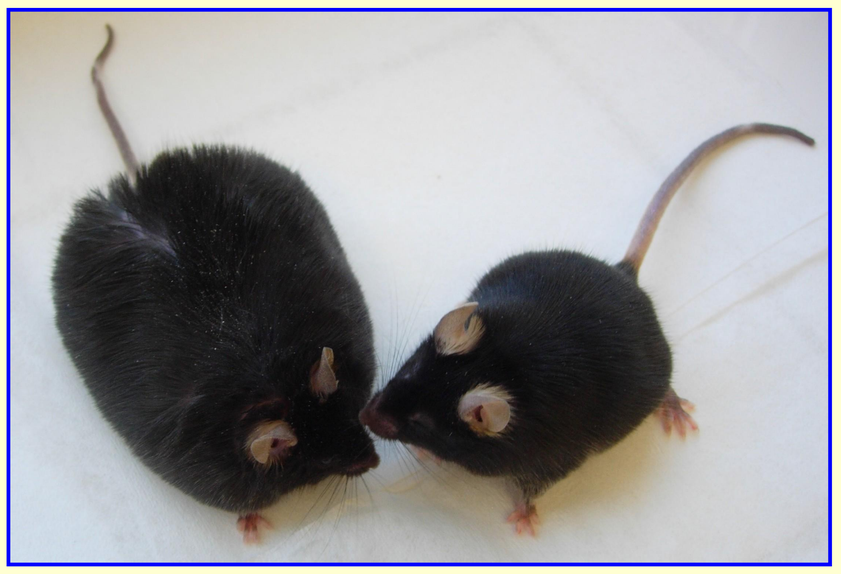
With the aid of our newly developed radiometric enzyme assays [2], we measured changes in activities of the key enzymes of TH metabolism, the three known iodothyronine deiodinases (IDs) in several depots of WAT, and also in BAT and liver of mice, maintained under the conditions that promoted either adipose tissue hypertrophy (i.e., during obesogenic treatment) or involution (after mild caloric restriction) [3].

Deiodinase of type 1 (D1) performs both, outer-ring 5'-monodeiodination and inner-ring 5-deiodination, converting prohormone thyroxine (T_4) either into biologically most active hormone 3,5,3'-triiodothyronine (T_3) or inactive 3,3',5'-triiodothyronine (reverse T_3), respectively. On the contrary, deiodinase of type 2 (D2) catalyzes specific 5'-deiodination, and deiodinase of type 3 (D3) specific 5-deiodination. Consequently, D3 inactivates both T_4 (producing rT_3) and T_3 (converting it into metabolite 3,3'-diiodothyronine T_2).

Principal iodothyronines, activated or inactivated by the iodothyronine deiodinases D1, D2 and D3



RESULTS



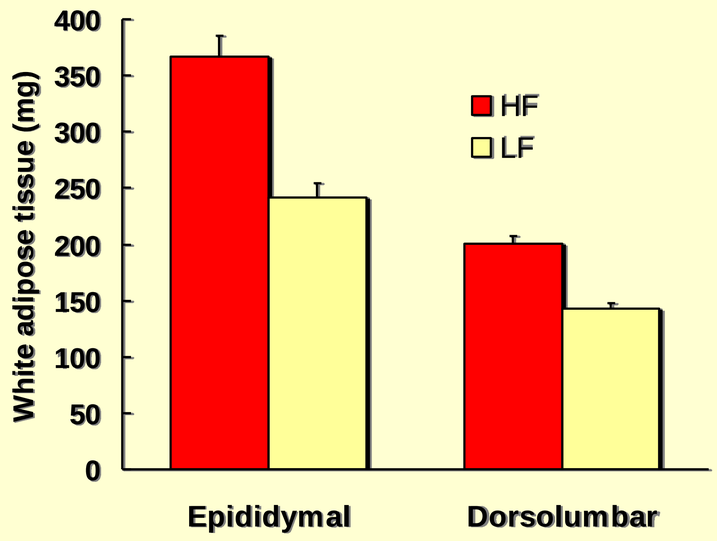


Fig. 1 Weight of epididymal and dorsolumbar fat depots in mice fed for two weeks HF- or LF-diet

Fig. 2 Obesity in the mouse induced by HF-diet feeding for eight weeks

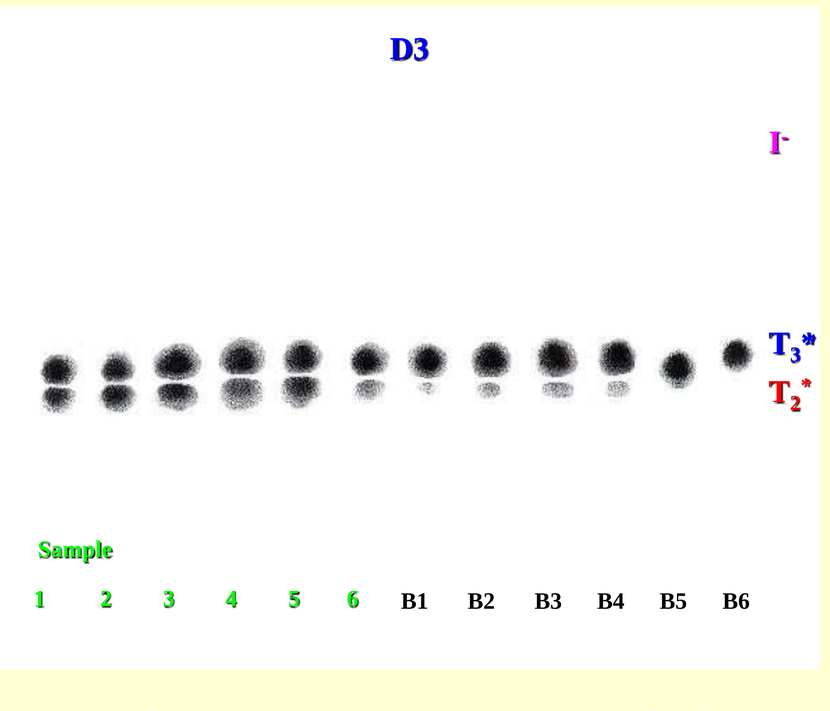


Fig. 3 Radiometric enzyme assay for D3: radiochromatogram of separated labeled product (T_2*) and non-radioactive iodide (I^-) from unconsumed substrate (T_3*)

Table 1 Mice growth characteristics and plasma levels of thyroid hormones in the course of obesogenic treatment (HF) and after caloric restriction (HF-CR). Data are means ± S.E.M. for two (n=18) and eight weeks (n=7-9) of obesogenic treatment and for five weeks of caloric restriction (n=11-12), *p < 0.05 for the effect of diet

Treatment/	2 weeks		8 weeks		5 weeks	
Parameter	LF	HF	LF	HF	HF-AL	HF-CR
Body weight gain (g)	3.3 ± 0.4	3.4 ± 0.6	9.0 ± 0.7	13.2 ± 1.5*	7.6 ± 0.4	2.6 ± 0.6*
Weight of fat depots (mg)						
EPI	242 ± 13	366 ± 22*	447 ± 63	1311 ± 320*	1913 ± 136	1402 ± 72*
DL	143 ± 4	200 ± 7*	180 ± 13	442 ± 80*	679 ± 45	475 ± 23*
BAT	105 ± 5	71 ± 3*	154 ± 8	137 ± 15	192 ± 10	139 ± 5*
Plasma levels of hormones						
Total T_4 (nmol/l)	36.9 ± 0.8	41.6 ± 1.1*	62.8 ± 4.0	62.9 ± 1.4	45.0 ± 4.7	54.6 ± 6.5
Total T_3 (nmol/l)	0.96 ± 0.03	1.30 ± 0.03*	0.71 ± 0.05	1.07 ± 0.05*	1.75 ± 0.08	1.75 ± 0.07
Free T_4 (pmol/l)	13.8 ± 0.6	13.2 ± 0.9	12.2 ± 0.8	11.3 ± 0.6	11.4 ± 0.7	11.6 ± 0.7
Free T_3 (pmol/l)	5.23 ± 0.54	4.90 ± 0.40	4.40 ± 0.55	4.21 ± 0.58	3.46 ± 0.18	2.65 ± 0.09*

Table 2 Changes in specific enzyme activity of D1 in mice adipose tissue depots and liver in the course of obesogenic treatment (HF) and after caloric restriction (HF-CR). Data are means ± S.E.M. for two (n=18) and eight weeks (n=7-9) of obesogenic treatment and for five weeks of caloric restriction (n=11-12), *p < 0.05 for the effect of diet

Treatment/	2 weeks		8 weeks		5 weeks	
Tissue	LF	HF	LF	HF	HF-AL	HF-CR
D1 activity (pmol T_2/h/mg prot.)						
EPI	5.6 ± 0.5	6.0 ± 1.0	3.5 ± 1.9	12.0 ± 1.1*	14.3 ± 1.6	7.2 ± 1.1*
DL	2.2 ± 0.3	3.8 ± 0.6*	6.5± 0.5	11.2 ± 1.3*	ND	ND
BAT	2.0 ± 0.3	2.1 ± 0.3	0.9 ± 0.2	0.8 ± 0.3	ND	ND
Liver	1288 ± 87	2178 ± 153*	877 ± 144	2364 ± 444*	2566 ± 281	1946 ± 202

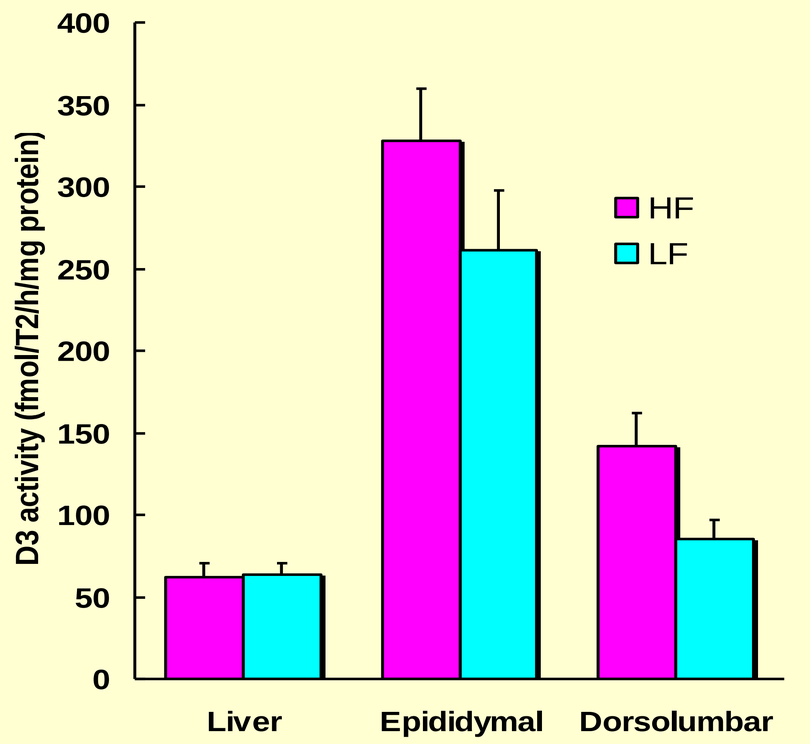


Fig. 4 D3 activity in the liver, epididymal and dorsolumbar fat in mice fed for two weeks HF- or LF-diet

CONCLUSIONS

- HF-diet feeding of mice resulted in a significantly higher weight of both epididymal-visceral and dorsolumbar-subcutaneous fat depots.
- Development of HF-diet-induced obesity in the mice was associated with stimulation of thyroid hormones metabolism and enhancement of D1 and D3 activities in WAT. However, D1 or D2 acti-vities in BAT did not change.
- Caloric restriction caused opposite changes in the metabolism of TH – this treatment decreased D1 activity in WAT, but not in the liver.
- The elaborated methods for radiometric determination of D1, D2 and D3 deiodinase activities proved to be extremely sensitive and rapid and, at the same time, reliable.

REFERENCES: [1] M.J. Obregon (2008) *Thyroid* 18: 185-195; [2] S. Pavelka (2010) *J. Radioanal. Nucl. Chem.* 286: 861-865; [3] Z. Macek Jílková, S. Pavelka et al. (2010) *Physiol. Res.* 59: 561-569

METHODS

Animals and treatment: 1) Obesogenic treatment - Male C57BL/6J mice were born and maintained at 30 ºC. At 4 weeks of age they were randomly assigned to a standard low-fat (LF) or a special high-fat (HF) diet and maintained on these diets for two or eight weeks before analysis. 2) Caloric restriction - Mice born and maintained at 22 ºC were fed the LF diet after weaning. Then, beginning at the age of three months, the mice were fed the HF diet for another seven weeks. During the last five weeks of the HF-feeding, one group of mice was fed *ad libitum* (HF-AL), while the other group was subjected to 10 % caloric restriction (HF-CR) compared with the HF-AL mice. Mice were killed and plasma, epididymal WAT and other tissues were collected and analyzed.

Biochemical analyses: Plasma levels of total T_4 and T_3 and free T_4 and T_3 were determined using commercial RIA kits (Immunotech, Beckman Coulter, Czech Republic). Our newly developed [2] radiometric enzyme assays for IDs (D1, D2 and D3) activities were based on the use of appropriate ^{125}I-labeled iodothyronines as substrates, TLC separation of the radioactive products (and/or non-radioactive iodide) from the unconsumed substrates and film-less autoradiography of radiochromatograms using storage phosphor screens. Quantification of the separated compounds was performed using a BAS-5000 laser scanner (Fujifilm Co., Japan).

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