European Thyroid Journal

Eur Thyroid J (DOI:10.1159/000381021)
(Accepted, unedited article not yet assigned to an issue)

Advanced Release: May 23, 2015

Received: December 12, 2014
Accepted after revision: February 16, 2015

High T_3, low T_4 serum levels in *Mct8*-deficiency are not caused by increased hepatic conversion through type I-deiodinase

Eva K. Wirth[1], Eddy Rijntjes[1], Franziska Meyer[1], Josef Köhrle[1], Ulrich Schweizer[1,2]

1 Institut für Experimentelle Endokrinologie, Charité-Universitätsmedizin Berlin, Berlin, Germany

2 present address: Institut für Biochemie und Molekularbiologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

Running title: Role of liver Dio1 activity in *Mct8*-deficiency

Contact:
Dr. Eva K. Wirth
eva.wirth@charite.de
Institut für Experimentelle Endokrinologie,
Charité-Universitätsmedizin Berlin,
Augustenburger Platz 1,
13353 Berlin, Germany
Phone: +49-30-450524105,
Fax: +49-30-450524922

Key words: Slc16a2, thyroid hormone, deiodinase, Allan-Herndon-Dudley syndrome, Dio1

Abstract

Background: The Allan-Herndon-Dudley syndrome is a severe psychomotor retardation accompanied by specific changes in circulating thyroid hormone levels (high T_3, low T_4). These are caused by mutations in the thyroid hormone transmembrane transport protein monocarboxylate transporter 8 (MCT8).

Objective: To test the hypothesis that circulating low T_4 and high T_3 levels are caused by enhanced conversion of T_4 via increased activity of hepatic type I-deiodinase (Dio1). *Methods:* We crossed mice deficient in *Mct8* with mice lacking Dio1 activity in hepatocytes. Translation of the selenoenzyme Dio1 was abrogated by hepatocyte-specific inactivation of selenoprotein biosynthesis. *Results:* Inactivation of Dio1 activity in the livers of global *Mct8*-deficient mice does not restore normal circulating thyroid hormone levels. *Conclusions:* Our data suggest that, although hepatic Dio1 activity is increased in *Mct8*-deficient mice, it does not cause the observed abnormal circulating thyroid hormone levels. Since global inactivation of Dio1 in *Mct8*-deficient mice does normalize circulating thyroid hormone levels, the underlying mechanism and relevant tissues involved remain to be elucidated.

Introduction

The monocarboxylate transporter 8 (Mct8) is the most specific thyroid hormone (TH) transmembrane transporter that is currently known. Mutations in MCT8 lead to a severe form of psychomotor retardation, the Allan-Herndon-Dudley syndrome [1]. Patients present with neurological symptoms including severe hypotonia, lack of speech and poor mental development. Specific endocrine abnormalities in circulating TH levels (low T_4, high T_3) in face of normal to elevated TSH levels paved the way to the discovery of underlying mutations in MCT8 in these patients [2,3]. Mouse models for *Mct8*-deficiency have been generated and replicate the endocrine phenotype seen in humans [4-6]. Low circulating T_4 and high circulating T_3 levels lead to the manifestation of local hypo- or hyperthyroidism in different organs and tissues depending on the presence of other TH transmembrane transporters. Tissues like liver [4,5], muscle [7] and kidney [8] are reportedly in a hyperthyroid state in *Mct8*-deficiency evaluated by deiodinase activities, while the brain rather shows signs of hypothyroidism measured by reduced uptake of T_3 into the brain and increased deiodinase 2 activity [4,5] or mixed hypo- and hyperthyroid changes assessed by behavioral analysis [9]. Until now, it is unclear what causes the low circulating T_4 and high T_3 concentrations. Several explanations have been suggested: *Mct8*-deficient mice demonstrate enhanced uptake and clearance of TH via the kidney possibly leading to a reduction of T_4 and T_3 in serum [8]. TH also accumulate in *Mct8*-deficient thyroid glands and are secreted at a slower rate upon TSH stimulation [10,11]. However, these findings do not seem to account for the low T_4 and elevated T_3 serum levels since they rather originate from enhanced metabolism of TH than from increased loss in the kidney or reduced secretion from the thyroid gland. Liao *et al.* determined in 2011 the consequences of combined *Mct8*- and *Dio1*- and/or *Dio2*-deficiency on the hypothalamus-pituitary-thyroid axis. They nicely demonstrated that the global deletion of *Dio1* in *Mct8*-deficient animals leads to a nearly complete normalization of circulating T_4 and T_3, as well as TSH levels [12]. It was therefore concluded that increased conversion of T_4 into T_3 by Dio1 is responsible for the elevated circulating T_3 and reduced T_4 levels in *Mct8*-deficiency. To directly test this hypothesis, we made use of our

previously described hepatocyte-specific selenoprotein-deficient mice (*Alb-Cre*;*Trsp*$^{fl/fl}$) that are devoid of deiodinase activity in hepatocytes [13]. Our model revealed that deletion of Dio1 activity in livers of *Mct8*-deficient mice has no major impact on circulating TH levels and is therefore not the underlying cause for the observed low T_4 and high T_3 serum levels in *Mct8*-deficiency.

Material and Methods

Animals

All animal experiments have been approved by the local authorities in Berlin, Germany and have been performed according to local regulations at the Charité-Universitätsmedizin Berlin, Germany. *Alb-Cre*;*Trsp*$^{fl/fl}$, as well as *Mct8*-deficient mice have been described before [9,13]. The data presented in this paper was generated using only male mice with the genotypes wt, *Mct8*$^{-/y}$, *Alb-Cre*;*Trsp*$^{fl/fl}$, and *Alb-Cre*;*Trsp*$^{fl/fl}$;*Mct8*$^{-/y}$. Matings were set up in a way to obtain animals of all genotypes as littermates.

Type I-deiodinase assay

Activities of the type I-deiodinase were determined in triplicate in liver homogenates (40µg protein/ml) based on an earlier described iodide release protocol [14] with slight modifications. Liver homogenates were incubated at 37°C for 60 minutes with 20 mM 1,4-dithiothreitol as cosubstrate, 0.3 µM nonradiolabeled rT_3 and ^{125}I-radiolabeled rT_3 (PerkinElmer, Hamburg, Germany; 0.82 µCi/pmol) in absence or presence of 1 mM propylthiouracil (PTU). The reaction was stopped by adding cold 10% BSA and 0.01 mM PTU and proteins were precipitated by adding 3 volumes of cold 10% trichloric acid. Samples were centrifuged and the supernatant was eluted over a Dowex-50 WX-2 column. The ^{125}I in the eluate was counted using a γ-counter (1277 Gammamaster, LKB Wallac, Turku, Finland). The absence or presence of H_2O or PTU in the reaction mixture differentiated between Dio1 activity and total deiodinase activity as the fraction of ^{125}I release blocked by PTU was assigned to Dio1.

TH assay

Total T_4 and T_3 levels were measured by competitive radioimmunoassays from DRG Instruments, Germany. Samples and calibrators for a standard curve were incubated with ^{125}Iod-T_4 or ^{125}Iod-T_3 as tracer in antibody-coated tubes for 1h. Bound radioactivity was determined in a gamma counter (1277 Gammamaster, LKB Wallac, Turku, Finland).

Results

Inactivation of hepatocyte-specific deiodinase activity in Mct8*-deficient mice*

At present, a mouse model for the conditional inactivation of the *Dio1* gene is not available. We therefore took advantage of the fact that deiodinases are selenoenzymes, i.e. enzymes carrying the rare amino acid selenocysteine (Sec). Incorporation of Sec depends on tRNA$^{(Sec)}$, which is encoded by the gene *Trsp*, of which a mouse model for the conditional inactivation is available. We have previously reported that hepatocyte-specific inactivation of selenoprotein translation abrogated hepatic deiodinase activity in *Alb-Cre*;*Trsp*$^{fl/fl}$ mice [13]. Expression of all selenoproteins is quantitatively abolished in livers of *Alb-Cre*;*Trsp*$^{fl/fl}$ mice [15]. Ablation of selenoprotein biosynthesis in hepatocytes does not lead to liver failure or other diseases [16,17]. Hence, we crossed global *Mct8*-deficient mice with our liver-specific deiodinase-deficient mice in order to test the hypothesis that hepatic deiodinase causes increased T_4 to T_3 conversion and subsequently low T_4, high T_3 serum levels in *Mct8*-deficiency [9,13]. All mice were apparently healthy, as body weight (bw) is not different between wt, *Mct8*$^{-/y}$ and *Alb-Cre*;*Trsp*$^{fl/fl}$ mice from our crossed mouse line. Only *Alb-Cre*;*Trsp*$^{fl/fl}$;*Mct8*$^{-/y}$ mice have a slightly reduced bw at the age of 2-3 months (Fig. 1A). Heart weights did not differ between groups when normalized for bw indicating no hypertrophic effect of TH on the heart in this age group (Fig. 1B). As expected, *Mct8*-deficient mice (*Mct8*$^{-/y}$) have a higher Dio1 activity in the liver than their littermate controls (Fig. 1C). Inactivation of deiodinases in control or *Mct8*-deficient mice reduced Dio1 activity to levels close to the detection limit (Fig. 1C). Residual Dio1 activity most likely stems from the very low amount of Dio1 that is expressed outside of hepatocytes in the liver, possibly in Kupffer cells.

Effect of hepatic Dio1-*deficiency in* Mct8-*deficient mice on circulating thyroid hormones*

Since combined global inactivation of *Dio1* and *Mct8* led to the normalization of circulating TH levels, we measured circulating levels of total T_4 and total T_3 to see the impact of hepatic *Dio1* inactivation on TH metabolism in global *Mct8*-deficient mice. Also in this combined mouse model, we can replicate the known endocrine phenotype of *Mct8*-deficiency with low T_4 and high T_3 levels in *Mct8*$^{-/y}$ as compared to littermate controls (Fig. 2). Inactivation of hepatic deiodinase activity led to only marginally increased total T_4 serum levels in *Mct8*-deficient mice. A slight increase in circulating T_4 levels upon hepatic Dio1 inactivation has been described before and may be related to reduced inactivation of T_4 [18]. Loss of Dio1 activity in *Mct8*-deficient livers does also not lead to a normalization of circulating T_3 levels (Fig. 2). They remain as high in *Alb-Cre*;*Trsp*$^{fl/fl}$;*Mct8*$^{-/y}$ mice as in *Mct8*$^{-/y}$ mice.

Discussion

High circulating T_3 concentrations in *MCT8*-deficient patients are considered to be responsible for increased energy expenditure and muscle wasting. At the same time, feeding the patients adequately is challenging, given their impaired motor capabilities, and weight loss often occurs. Serum TH constellations with high T_3 and low T_4 concentrations in *MCT8*-deficient patients are considered to be responsible for a variety of these peripheral phenotypes through hyperthyroid states in MCT8 independent tissues like skeletal muscle and liver. Lowering serum T_3 may thus represent a therapeutic goal, but this is difficult to reach in the presence of abnormally low T_4 levels in the patients. Local conversion of T_4 to T_3 is the major source of cerebral T_3. Therefore, treatments potentially lowering T_4 are at risk of further reducing cerebral TH uptake and T_3 availability. It is thus a pertinent question how these altered serum TH levels are caused.

Although a variety of data has been collected in mouse models of *Mct8*-deficiency, the mechanism for the manifestation of these altered serum TH levels is still unclear. Loss of TH through the kidney was proposed [8]. How increased total T_3 could be maintained while T_4 is selectively lost is difficult to envision at present. Reduced secretion of TH from the thyroid

gland itself was also proposed [10,11]. How the release of T_4 could be lowered while at the same time T_3 release would be increased from the thyroid gland is again not clear. Moreover, a patient with a mutation in MCT8 treated with levothyroxine after complete thyroidectomy maintained the high T_3, low T_4 levels in serum [6]. Increased conversion of T_4 to T_3 by deiodinases is thus a possible explanation. Combined deletion of *Mct8* and *Dio1* in mice resulted in a normalization of serum TH parameters and subsequent improvement of brain T_3 content [12]. In contrast, genetic inactivation of *Dio2* in *Mct8*-deficient mice did not improve TH serum concentrations and, on the contrary increased changes in brain gene expression. These data together suggested that peripheral conversion of T_4 to T_3 via Dio1 may establish the high T_3, low T_4 hormonal constellation in *Mct8*-deficiency. Since increased access of T_3 to the liver does not depend on Mct8 and further stimulates Dio1 expression, hepatic Dio1-mediated conversion of T_4 represented a plausible mechanism of establishing the abnormal TH levels outlined before. Nonetheless, our data presented here appear to refute this attractive hypothesis. Targeted inactivation of hepatic Dio1 activity neither normalized T_3 nor T_4 levels in *Mct8*-deficient mice. The mild increase in serum T_4 levels in *Alb-Cre*;*Trsp*$^{fl/fl}$;*Mct8*$^{-/y}$ mice, which is also seen in *Alb-Cre*;*Trsp*$^{fl/fl}$ mice rather hints to reduced T_4 degradation as in Dio1$^{-/-}$ mice because it does not alter T_3 levels. Whether increased Dio1 activity in other organs like kidney or other mechanisms underlie the abnormal TH serum concentrations will be a matter of future studies. The genesis of the abnormal TH constellation in serum upon *Mct8*-deficiency still remains an open question.

Acknowledgements

The authors would like to thank Gabriele Böhm and Antje Kretschmer for excellent technical assistance. Conditional *Trsp* knockout mice were kindly provided by Brad Carlson and Dr. Dolph Hatfield, Molecular Biology of Selenium, Basic Research Laboratory, NCI, NIH, Bethesda, MD, USA. Funding for this project has been provided by Deutsche Forschungsgemeinschaft (WI3768/1-1 and WI3768/2-1 Thyroid Trans Act).

Conflict of interest

The authors declare no conflict of interest.

References

1 Allan W, Herndon CN, Dudley FC: Some examples of the inheritance of mental deficiency: Apparently sex-linked idiocy and microcephaly. Am J Ment Defic 1944;48:325-334.
2 Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Barrett TG, Mancilla EE, Svensson J, Kester MH, Kuiper GG, Balkassmi S, Uitterlinden AG, Koehrle J, Rodien P, Halestrap AP, Visser TJ: Association between mutations in a thyroid hormone transporter and severe x-linked psychomotor retardation. Lancet 2004;364:1435-1437.
3 Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S: A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. Am J Hum Genet 2004;74:168-175.
4 Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, Raivich G, Bauer K, Heuer H: Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. J Clin Invest 2007;117:627-635.
5 Dumitrescu AM, Liao XH, Weiss RE, Millen K, Refetoff S: Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (mct) 8-deficient mice. Endocrinology 2006;147:4036-4043.
6 Wirth EK, Sheu SY, Chiu-Ugalde J, Sapin R, Klein MO, Mossbrugger I, Quintanilla-Martinez L, Hrabě de Angelis M, Krude H, Riebel T, Rothe K, Köhrle J, Schmid KW, Schweizer U, Grüters A: Monocarboxylate transporter 8 deficiency: Altered thyroid morphology and persistent high triiodothyronine/thyroxine ratio after thyroidectomy. Eur J Endocrinol 2011;165:555-561.
7 Di Cosmo C, Liao XH, Ye H, Ferrara AM, Weiss RE, Refetoff S, Dumitrescu AM: Mct8-deficient mice have increased energy expenditure and reduced fat mass that is abrogated by normalization of serum t3 levels. Endocrinology 2013;154:4885-4895.
8 Trajkovic-Arsic M, Visser TJ, Darras VM, Friesema EC, Schlott B, Mittag J, Bauer K, Heuer H: Consequences of monocarboxylate transporter 8 deficiency for renal transport and metabolism of thyroid hormones in mice. Endocrinology 2010;151:802-809.
9 Wirth EK, Roth S, Blechschmidt C, Hölter SM, Becker L, Racz I, Zimmer A, Klopstock T, Gailus-Durner V, Fuchs H, Wurst W, Naumann T, Bräuer A, Hrabě de Angelis M, Köhrle J, Grüters A, Schweizer U: Neuronal 3',3,5-triiodothyronine (t3) uptake and behavioral phenotype of mice deficient in mct8, the neuronal t3 transporter mutated in allan-herndon-dudley syndrome. J Neurosci 2009;29:9439-9449.
10 Trajkovic-Arsic M, Muller J, Darras VM, Groba C, Lee S, Weih D, Bauer K, Visser TJ, Heuer H: Impact of monocarboxylate transporter-8 deficiency on the hypothalamus-pituitary-thyroid axis in mice. Endocrinology 2010;151:5053-5062.
11 Di Cosmo C, Liao XH, Dumitrescu AM, Philp NJ, Weiss RE, Refetoff S: Mice deficient in mct8 reveal a mechanism regulating thyroid hormone secretion. J Clin Invest 2010;120:3377-3388.
12 Liao XH, Di Cosmo C, Dumitrescu AM, Hernandez A, Van Sande J, St Germain DL, Weiss RE, Galton VA, Refetoff S: Distinct roles of deiodinases on the phenotype of mct8 defect: A comparison of eight different mouse genotypes. Endocrinology 2011;152:1180-1191.
13 Streckfuss F, Hamann I, Schomburg L, Michaelis M, Sapin R, Klein MO, Köhrle J, Schweizer U: Hepatic deiodinase activity is dispensable for the maintenance of normal circulating thyroid hormone levels in mice. Biochem Biophys Res Commun 2005;337:739-745.

14 Leonard JL, Rosenberg IN: Iodothyronine 5'-deiodinase from rat kidney: Substrate specificity and the 5'-deiodination of reverse triiodothyronine. Endocrinology 1980;107:1376-1383.
15 Seeher S, Atassi T, Mahdi Y, Carlson BA, Braun D, Wirth EK, Klein MO, Reix N, Miniard AC, Schomburg L, Hatfield DL, Driscoll DM, Schweizer U: Secisbp2 is essential for embryonic development and enhances selenoprotein expression. Antioxid Redox Signal 2014
16 Schweizer U, Streckfuss F, Pelt P, Carlson BA, Hatfield DL, Köhrle J, Schomburg L: Hepatically derived selenoprotein p is a key factor for kidney but not for brain selenium supply. Biochem J 2005;386:221-226.
17 Sengupta A, Carlson BA, Weaver JA, Novoselov SV, Fomenko DE, Gladyshev VN, Hatfield DL: A functional link between housekeeping selenoproteins and phase ii enzymes. Biochem J 2008;413:151-161.
18 Schneider MJ, Fiering SN, Thai B, Wu SY, St Germain E, Parlow AF, St Germain DL, Galton VA: Targeted disruption of the type 1 selenodeiodinase gene (dio1) results in marked changes in thyroid hormone economy in mice. Endocrinology 2006;147:580-589.

Figure 1

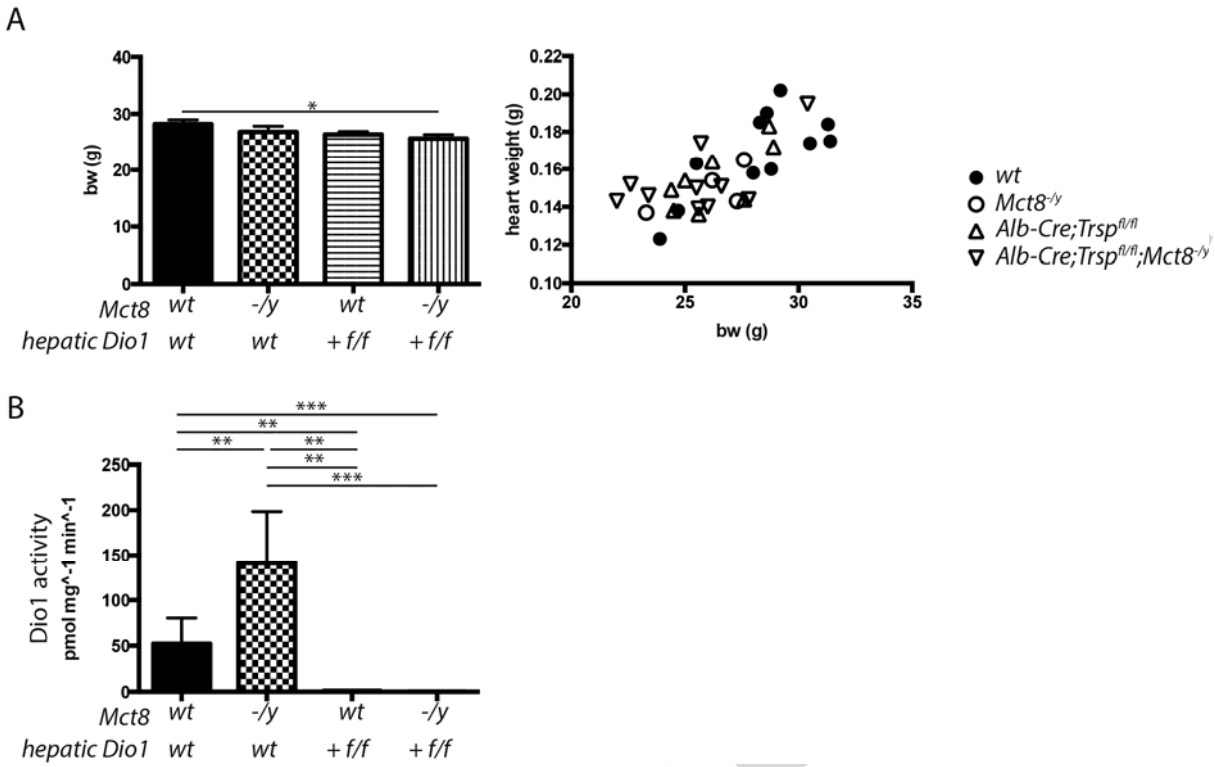

Figure 1: Inactivation of deiodinase activity in *Mct8*-deficient mice. (A) Wild-type (wt), *Mct8*-deficient (*Mct8*$^{-/y}$) and hepatocyte-specific deiodinase-deficient (*Alb-Cre;Trsp*$^{fl/fl}$) mice have normal body weight (bw). Only double-knockout hepatocyte-specific deiodinase- and global *Mct8*-deficient (*Alb-Cre;Trsp*$^{fl/fl}$*;Mct8*$^{-/y}$) mice showed a slightly lower bw compared to control littermates. Heart weight normalized for bw did not differ between the analyzed groups. (B) Measuring liver type I-deiodinase activity in all groups lead to the expected increase in *Mct8*-deficient mice. Inactivation of deiodinase activity in wt or in *Mct8*-deficent livers results in a nearly abolished activity. Data are presented as means ± SEM. *, P < 0,05; **, P < 0,01; ***, P < 0,001 (Mann-Whitney-U-test).

Figure 2

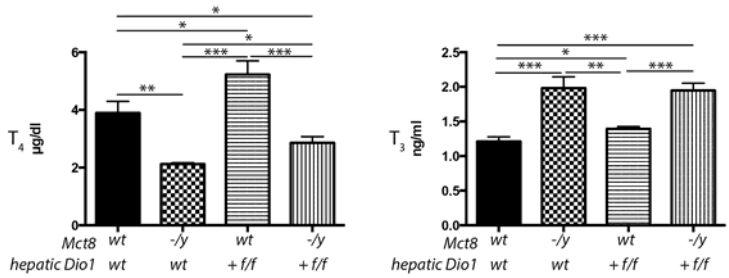

Figure 2: Loss of deiodinase activity in *Mct8*-deficient livers does not normalize abnormal circulating TH levels. Total circulating T_4 and T_3 level were measured in wt, *Mct8*$^{-/y}$, *Alb-Cre;Trsp*$^{fl/fl}$ and *Alb-Cre;Trsp*$^{fl/fl}$*;Mct8*$^{-/y}$ mice. Loss of *Mct8* leads to the expected low T_4 and increased T_3 levels in serum. Inactivation of hepatic deiodinase only marginally increased the T_4 and T_3 level. *Alb-Cre;Trsp*$^{fl/fl}$*;Mct8*$^{-/y}$ mice display also a minor increase in T_4, while circulating T_3 levels were not normalized compared to *Mct8*-deficient mice. Data are presented as means ± SEM. *, P < 0,05; **, P < 0,01; ***, P < 0,001 (Mann-Whitney-U- test)

European Thyroid Journal *(Official Journal of the European Thyroid Association)*
Journal Editor: Wiersinga W.M. (Amsterdam)
ISSN: 2235-0640 (Print), eISSN: 2235-0802 (Online)
www.karger.com/ETJ