Transthyretin is not necessary for thyroid hormone metabolism in conditions of increased hormone demand

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Abstract

Thyroid hormones circulate in blood mainly bound to plasma proteins. Transthyretin is the major thyroxine plasma carrier in mice. Studies in transthyretin-null mice revealed that the absence of transthyretin results in euthyroid hypothyroxinemia and normal thyroid hormone tissue distribution, with the exception of the choroid plexus in the brain. Therefore, transthyretin does not influence normal thyroid hormone homeostasis under standard laboratory conditions. To investigate if transthyretin has a buffer/storage role we challenged transthyretin-null and wild-type mice with conditions of increased hormone demand: (i) exposure to cold, which elicits thermogenesis, a process that requires thyroid hormones; and (ii) thyroidectomy, which abolishes thyroid hormone synthesis and secretion and induces severe hypothyroidism. Transthyretin-null mice responded as the wild-type both to changes induced by stressful events, namely in body weight, food intake and thyroid hormone tissue content, and in the mRNA levels of genes whose expression is altered in such conditions. These results clearly exclude a role for transthyretin in thyroid hormone homeostasis even under conditions of increased hormone demand.

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Introduction

Transthyretin (TTR) is synthesized by the liver and the choroid plexus (Harms et al. 1991) and acts as the major rodent plasma (Davis et al. 1970) and cerebrospinal fluid (Hagen & Solberg 1974) carrier for thyroxine (T\textsubscript{4}) and the retinol-binding protein–retinol complex. TTR synthesis is a phylogenetically conserved event (Harms et al. 1991), and starts early during embryonic development. It has for a long time been suggested that TTR is involved in thyroid hormone homeostasis and hormone delivery to the brain (Dickson et al. 1987, Dratman et al. 1991, Chanoine et al. 1992). However, studies in a TTR–null mouse strain revealed that despite a 50\% decrease in total T\textsubscript{4} plasma levels (Episkopou et al. 1993), no alterations are found in circulating free T\textsubscript{4} or total and free triiodothyronine (T\textsubscript{3}) levels (Palha et al. 1994), and that TTR is not necessary for thyroid hormones to be normally distributed both to and within tissues (Palha et al. 1997, 2000, 2002). These studies, together with observations in other carrier protein deficiencies such as that of T\textsubscript{4}-binding globulin (TBG) in humans (Refetoff 1989) and albumin deficiency in rats (Mendel et al. 1989) support the free hormone hypothesis for thyroid hormones. This hypothesis states that the biologically important pool of hormone in the circulation is in the free form (Mendel 1989). The role of carrier proteins in hormone delivery to tissues has been a matter of great debate (Schreiber et al. 1990, Palha 2002, Schreiber 2002). Individually, transport proteins might be redundant and part of a back-up mechanism for other carriers; they might not be essential but still contribute to hormone delivery to particular tissues, or they might represent important storage/buffer reservoirs required under conditions of increased or decreased hormone demand. Therefore, exposure to stressful conditions might provide further information on the physiological role of individual proteins.

Thyroid hormones are essential for the proper development of the central nervous system and regulate many different functions both during development and in the adult organism. Among these is the regulation of basal cellular metabolism (Silva 2003). The majority of thyroid hormone actions are mediated by the transcriptional activation or repression of target genes. T\textsubscript{3}, the biologically...
active hormone, derived from circulating T₃ or from local deiodination of T₄ by deiodinas, interacts with nuclear receptors that bind to response elements in target genes (Ribeiro et al. 1995). Deiodinase enzymes are, therefore, important modulators of thyroid hormone action, and respond (both at the mRNA and protein and activity levels) in accordance with altered thyroid hormone availability (Bianco et al. 2002).

Exposure to cold induces a physiological adaptation of the basal cellular metabolism (Pecqueur et al. 2001), in which there is an increased rate of aerobic cellular metabolism and the production of heat by adaptive (or facultative) thermogenesis. Heat production is accomplished by the activation of uncoupling proteins that dissipate the proton gradient across the inner mitochondrial membrane (Nedergaard et al. 2001). In rodents, brown adipose tissue (BAT) is the major site of adaptive thermogenesis. BAT responsiveness to cold, initiated by norepinephrine (NE), elicits an increase in cAMP that results in the activation of several genes including those of uncoupling protein 1 (UCP1) (Bouillaud et al. 1984, Silva & Rabelo 1997) and deiodinase type 2 (DII) (Silva & Larsen 1983, Klingenspor 2003). Augmented T₃ resulting from increased DII activity also contributes to regulation of heat production by uncoupling proteins, enzymes of the basal cell metabolism and elements of the NE signaling pathway (Silva & Larsen 1983, Ribeiro et al. 2000, Silva 2001, Klingenspor 2003).

Extreme thyroid hormone deficiency is induced by ablation of thyroid tissue. Thyroid hormone plasma content decreases to less than 5% of normal values in 15 days after thyroidectomy in rats, but T₄ and T₃ are still detected in tissues after 4 months (Obregon et al. 1981). It appears reasonable to assume that the circulating pool of hormone bound to a carrier protein, namely to TTR, contributes to the amount of serum thyroid hormones after thyroidectomy.

In order to investigate whether TTR has a storage/buffer role in thyroid hormone homeostasis, we challenged TTR-null mice to cold exposure and removal of the thyroid gland, conditions of moderate and extremely increased hormone demand respectively.

Materials and Methods

Animals

All experiments were conducted using 1-month-old wild-type or TTR-null mice (Episkopou et al. 1993), in accordance with National and European Union guidelines for the care and handling of laboratory animals. Mice, under 12 h light cycles, were given chow and tap water freely.

Exposure to cold

Control animals kept at 23 ± 1 °C and cold-exposed animals kept at 4 ± 2 °C were individually housed and provided with minimal bedding material. Daily body weight and food intake were registered. Animals, fasted overnight, were killed by rapid decapitation 1 month after the onset of exposure to cold; serum and tissue samples of liver, kidney, interscapular BAT and brain (excluding cerebellum and brainstem) were rapidly frozen in dry ice and stored at −80 °C until analysis. Separate aliquots of liver and BAT samples were collected for RNA extraction.

Thyroidectomy

Total thyroid gland removal or sham surgeries were performed under ketamine (150 mg/kg) plus medetomidine (0·3 mg/kg) i.p. anesthesia. Since removal of thyroid follicles in adult rats by surgical thyroidectomy is not always totally successful, thyroidectomized animals received, 1 week later, 100 μCi ¹³¹I to ensure complete removal of thyroid tissue. One week was usually left between the surgery and the radio-thyroidectomy, so that plasma thyroid-stimulating hormone (TSH) had time to increase and to ensure maximal uptake by remnants of the radioiodine. This, however, imposes some restrictions on the experimental procedures, because destruction of thyroid remnants and excretion of ¹³¹I-labeled compounds from the body is not immediate and ¹³¹I in serum and tissues may interfere with later determinations by RIA. This is the reason for waiting until a week before killing the animals. Moreover, previous work (Obregon et al. 1981) indicates that disappearance of T₄ and T₃ from plasma at 2 weeks after thyroidecomy occurs rapidly, as expected from the short biological half-lives, but both iodothyronines may still be found in many tissues. Because the parathyroids were removed along with the thyroid gland, thyroidectomized animals received 2% calcium lactate in their drinking water. Either 1 or 2 weeks after ¹³¹I injection, animals were anesthetized as described above, and heparin 0·17% (20–30 μl) was administered through the vena cava prior to blood withdrawal, followed by perfusion with 20 ml 0·05 M pH 7·4 phosphate buffer containing 0·9% NaCl. Samples of plasma, liver, kidney, heart, cortex and cerebellum were collected, frozen and stored as described above. To assess complete thyroid removal, animals subjected to this procedure were considered only if the weight gain between the iodine injection and the last day of experiment was less than 15% of their initial body weight, and if plasma TSH values were markedly increased.

TSH determination

TSH plasma levels were measured using the specific RIAs developed for the rat provided by the Rat Pituitary Agency of the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK, NIH, Bethesda, MD, USA). Results were expressed in weight equivalents of the NIDDK r-TSH RP-3 preparation.
**Thyroid hormone determination**

T4 and T3 were measured in all samples after extraction and purification of the iodothyronines, as previously described (Morreale de Escobar et al. 1985, 1994). In brief, methanol was added to the frozen sample and it was homogenized. Tracer amounts of $[^{131}I]T_4$ and $[^{125}I]T_3$, were added to each homogenate, followed by extraction of endogenous and added tracers with chloroform–methanol (2:1). The iodothyronines were then back-extracted into an aqueous phase and purified using AG 1 × 2 resin columns (Bio-Rad, Hercules, CA, USA). After a pH gradient, the iodothyronines were eluted with 70% acetic acid and evaporated to dryness. Each extract was counted using a gamma counter. Aliquots of the PCR products (10 µl) were separated by 2% agarose gel electrophoresis and stained with ethidium bromide. Gels were visualized with AlphaImager 2200 (AlphaInnotech, San Leandro, CA, USA) and analyzed densitometrically with the corresponding AlphaEase software. The expression level of the housekeeping gene HPRT was used as an internal standard, to which other PCR amplification products were normalized.

In another set of experiments, we confirmed the data obtained with the Quantum RNA 18S internal standards (Ambion, Austin, TX, USA) as housekeeping gene.

**RNA extraction and semiquantitative RT-PCR**

Total RNA was isolated from liver and BAT using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. cDNA synthesis was performed using the Superscript First-Strand Synthesis System for RT-PCR (Invitrogen) and semiquantitative multiplex PCR reactions were performed as previously described (Wong et al. 1994). Briefly, each PCR cycle was composed of the following steps: 94 °C for 30 s, 60 °C for 45 s and 72 °C for 60 s. A sequential series of PCR reactions using each primer pair was performed initially to determine the number of cycles in which the amplification would reside within the exponential phase of the amplification curve for both the gene under study and the housekeeping gene, hypoxanthine guanine phosphoribosyl transferase (HPRT). In accordance we chose 27, 26, 30 and 25 cycles for deiodinase type 1 (D1), DII, TBG or UCP1 respectively. Using an established ‘primer-dropping’ method (Wong et al. 1994), 21 cycles were used to amplify the HPRT gene in the same reaction in which the gene under study was amplified.

The oligonucleotide primers for D1, DII, TBG, UCP1 and HPRT were synthesized using the Primer3 software (Rozen & Skaletsky 2000) on the basis of the following GenBank sequences: AY575783 (TBG); NM007860 (D1); NM010050 (DII); U63419 (UCP1); XM356404 (HPRT). The sequences of oligonucleotide primers were:

- D1 sense, CTGGAAAAGCTTTGCACTCC, D1 antisense, AGGGTACACTCTGGATTGG;
- DII sense, ATGGGACCTCCTAGCGTAGACTTG, DII antisense, TGAACCAAAGTGGACCCACC;
- UCP1 sense, GTCTTAGGGACCACCTACCA, UCP1 anti-sense, CCCGTGTAGCGGGGTTT;
- HPRT sense, GCTGGTGAACGCTCT, HPRT anti-sense, CACAGGACTAGAACCCTTG;
- TBG sense, CAACAGGGGCTTCAACATT, TBG anti-sense, TGGTGCTCTTG TCCACTGAG.

Aliquots of the PCR products (10 µl) were separated by 2% agarose gel electrophoresis and stained with ethidium bromide. Gels were visualized with AlphaImager 2200 (AlphaInnotech, San Leandro, CA, USA) and analyzed densitometrically with the corresponding AlphaEase software. The expression level of the housekeeping gene HPRT was used as an internal standard, to which other PCR amplification products were normalized.

In another set of experiments, we confirmed the data obtained with the Quantum RNA 18S internal standards (Ambion, Austin, TX, USA) as housekeeping gene.

**Statistics**

Body weight and food intake comparisons were made using Student’s t-test with P < 0.05 considered as statistically significant. T4 and T3 levels were compared by two-way (strain and exposure to cold) ANOVA, after testing for homogeneity of variance by Levene’s test. Logarithmic transformation ensured homogeneity of variance when this was not found with the raw data. The F statistic was considered significant at P < 0.05.

Because removal of the thyroid results in a drastic reduction of thyroid hormone levels, it is not possible to apply a parametric statistical test that includes both the results from the sham-operated animals and those of the thyroidectomized ones. Therefore, we chose to compare, independently, the effect of thyroid removal in each experimental group (TTR-null vs wild-type) and the two strains within each experimental condition (sham or thyroidectomy), using Student’s t-test.

All values presented are means ± s.d. from four to nine samples per group. Statistical analyses were performed with SPSS version 12·0 for Windows (SPSS, Inc., Chicago, IL, USA).

**Results**

**Effects of cold exposure on thyroid hormone metabolism**

**Body weight and food intake** We chose 1-month-old animals because they were expected to grow rapidly during the 1-month duration of the experiment, and an effect of the absence of TTR could be more easily disclosed. At the beginning of the experiment (day zero), wild-type and TTR-null mice had similar body weights both in the groups maintained at room temperature.
Thyroid hormone concentrations The effects of exposure to cold on thyroid hormone concentrations are presented in Fig. 1.

Except for the liver, exposure to cold did not induce changes in T₄ tissue content. In the liver, both wild-type and TTR-null mice presented a cold-induced reduction in T₄ levels (F=27.532, P<0.001). A slight decrease in T₄ kidney levels, which was not sufficient to result in a cold-induced statistically significant effect, was more pronounced in the TTR-null mice (F=8.196, P<0.01).

TTR total serum T₄ was strongly reduced in TTR-null mice both at room temperature and when mice were exposed to cold (F=4.562, P=0.05).
The slight difference in total T₄ brain levels in animals kept at room temperature seemed to be attenuated by exposure to cold.

Exposure to cold resulted in increased T₃ concentrations in all organs studied, and this was identical for both wild-type and TTR-null mice. However, in the serum this effect was not as pronounced in the TTR-null mice, since we observed a strain effect (F=9·899, P<0·01). T₃ derives mainly from local T₄ deiodination. Interestingly, and in accord with the lower T₄ levels we found in the kidney of cold-exposed TTR-null mice, the increase in T₃ induced by exposure to cold was less pronounced in the TTR-null mice (F=4·996, P<0·05).

A summary of the ANOVA is presented in Table 1.

**Table 1 Two-way ANOVA statistics for T₄ and T₃ concentrations in wild-type and TTR-null mice maintained at room temperature or after a 1 month exposure to cold**

<table>
<thead>
<tr>
<th></th>
<th>Strain effect</th>
<th>Effect of exposure to cold</th>
<th>Strain vs exposure to cold interaction</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>T₄</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>45·521</td>
<td>&lt;0·001</td>
<td>0·344</td>
</tr>
<tr>
<td>Liver</td>
<td>0·452</td>
<td>NS</td>
<td>27·352</td>
</tr>
<tr>
<td>Kidney</td>
<td>8·196</td>
<td>&lt;0·01</td>
<td>0·156</td>
</tr>
<tr>
<td>Brain</td>
<td>1·069</td>
<td>NS</td>
<td>0·150</td>
</tr>
<tr>
<td>T₃</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>9·899</td>
<td>&lt;0·001</td>
<td>23·390</td>
</tr>
<tr>
<td>Liver</td>
<td>3·973</td>
<td>NS</td>
<td>35·258</td>
</tr>
<tr>
<td>Kidney</td>
<td>4·996</td>
<td>&lt;0·05</td>
<td>40·069</td>
</tr>
<tr>
<td>Brain</td>
<td>0·331</td>
<td>NS</td>
<td>43·709</td>
</tr>
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</table>

NS, not-statistically significant.

Expression levels of DI, DII, TBG and UCP1

Animals left in the cold presented a 2·5-fold increase in the expression of liver DI (P<0·05) (Fig. 2a). This increase was similar in both wild-type and TTR-null mice.

The BAT mRNA levels of DII (Fig. 2b) were, as expected, increased by exposure to cold. As seen on Fig. 2c, after 1 month at 4°C, a statistically significant increase in the mRNA for UCP1 was similarly observed in wild-type and TTR-null mice.

In order to investigate whether TBG could compensate for the absence of TTR in the cold, we measured the levels of liver TBG mRNA. TBG mRNA was similarly down-regulated (P<0·05) by cold, in TTR-null and wild-type mice, five and four times respectively. No statistical differences were seen for the TBG expression levels between TTR-null and wild-type mice at room temperature or in the cold.

**Effects of thyroidectomy on thyroid hormone metabolism in TTR-null mice**

**Thyroid hormone concentrations** In the absence of thyroid, there is no synthesis or secretion of thyroid hormones, and in such animals we can assess the possible role of TTR in mobilizing existing hormone stores. The weight gain of the thyroidectomized mice between the radiiodine injection and the last day of experiment was less than 15% of their initial body weight; plasma TSH values were markedly increased (>16 ng/ml) after thyroidectomy and were clearly indicative of a hypothyroid state.

Two (Fig. 3) or 3 weeks (data not shown) after removal of the thyroid gland there was a drastic and similar reduction in T₄ and T₃ plasma and tissue levels, whether TTR was present or absent. While for the plasma T₄, the strain effect continued to be present when the thyroid was ablated, for the heart, a smaller T₃ decrease was observed in the TTR-null mice (F=12·540, P<0·01). A summary of the statistical analysis is presented on Table 2.

**Expression levels of TBG** Thyroidectomy resulted in the up-regulation (P<0·05) of liver TBG mRNA in both TTR-null and wild-type mice (1·7 and 1·5 times respectively); no differences were seen between TTR-null and wild-type mice either in the sham or in the thyroidectomized groups.

**Discussion**

Previous studies from our own and other laboratories have failed to establish a limiting role for TTR in ensuring adequate supplies of thyroid hormone to mouse tissues under conditions of normal laboratory rearing. The present work was carried out in an effort to address the question of whether, or not, there is a requirement for TTR when pathophysiological conditions impose increased demand for thyroid hormone availability to tissues. Surprisingly, the results did not show any major difference between the response of TTR-null and wild-type mice during exposure to cold or during hypothyroidism caused by thyroidectomy.

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We have previously shown that TTR is not required for normal T4 tissue uptake or distribution, and its absence does not impair hormone tissue contents, despite the low circulating levels of total T4 (Palha et al. 1994, 1997). Even the absence of TTR from the choroid plexus, where it represents the major protein synthesized, does not influence the proper access and distribution of T4 within the brain (Palha et al. 2000). Considering that the TTR-null mice have normal free thyroid hormone levels, the data obtained so far from studying these mice are in agreement with the free hormone hypothesis (Mendel 1989), as previously discussed (Palha et al. 1994).

However, carrier proteins are believed to have buffer/reservoir roles (Schreiber 2002), and this should become apparent if conditions of increased hormone demand are imposed. Exposure to cold represents a physiological situation of increased cellular metabolic activity for which thyroid hormones are required.

We chose 1-month-old animals because they were expected to grow rapidly during the 1-month duration of the experiment, and an effect of the absence of TTR could be more easily observed. In the cold, part of the reducing equivalents derived from fuel oxidation are lost to produce heat. Therefore, adaptation to cold includes an increase in food intake and, at least in the initial phases, increased mobilization of stored energy. If TTR-bound T4 were important in the cold-induced increased demand for thyroid hormones, we would expect the TTR-null mice to adapt poorly or perhaps fail to survive at all in a cold environment. This was not the case. TTR-null mice adapted as well as the wild-type mice to a 1 month exposure to 4°C: both genotypes responded with an increase in food intake and changes in serum and tissue thyroid hormone content. In addition, body weight gain was also similar in the two groups of animals.

The increased need for thyroid hormone induced by cold is reflected in the elevation of serum and tissue T3, which we show here to be similar for TTR-null and wild-type mice. T3, the biologically active thyroid hormone, derives mainly from the local deiodination of T4 (Silva & Larsen 1985, Bianco et al. 2002). This explains the observed decrease in T4 tissue content and increase in T3 content associated with exposure to cold, especially in the liver, which produces a major fraction of circulating T3 (Bianco et al. 2002). In accord, we observed an increase in the liver mRNA levels of DI after 1 month of cold exposure. The facts that kidney T3 did not increase in the TTR-null as much as in the wild-type and that circulating T3 mainly derives from thyroid and peripheral (liver and kidney) T4 deiodination might explain that in the cold-exposed animals the increase in serum T3 in the TTR-null was not as pronounced as in the wild-type mice.

Interestingly, exposure to cold did not induce changes in T4 brain content within each genotype, despite the increase seen for T3. There is controversy on the effect of cold on the activity of brain DII (Silva & Larsen 1983, Anguiano et al. 1995, Sullo et al. 2003). Whether the increase in T3 observed by exposure to cold results from increased T4 and/or T3 delivery to the brain or altered DII and/or DIII activities remains to be elucidated. In any case, it is interesting to note that the slight, but statistically significant lower T4 content found in the TTR-null brain at room temperature is not aggravated, but rather...
Figure 3 Effect of thyroidectomy on $T_4$ and $T_3$ serum (ng/ml) and tissue (ng/g wet weight) content of wild-type and TTR-null mice. Two weeks after complete ablation of the thyroid gland both TTR-null and wild-type mice presented drastic reductions in circulating and tissue $T_4$ and $T_3$ levels. Statistical analysis is summarized in Table 2. Sham, sham surgery; Tx, thyroidectomized.
disappears, upon exposure to cold. It is possible that in the absence of a carrier/buffer protein such as TTR in the choroid plexus, and in conditions of increased hormone demand, T₄ has more ready access to the brain via the blood–choroid plexus–CSF route.

Thyroid hormones are important for adaptive thermogenesis in BAT. Adaptation to cold is mediated by production of heat, a process that is accomplished by the dissipation of the protein gradient by the BAT-specific mitochondrial protein UCP1. By inducing the expression of UCP1, both T₃ and NE (Bianco et al. 1988) contribute to the maintenance of a normal thermal condition. UCP1-induced activation by T₃ is, in itself, indirectly dependent on sympathetic stimulation of DII (Klingenspor 2003). The absence of TTR did not influence the expected increase in BAT DII mRNA upon exposure to cold. With respect to UCP1, acute (Bianco et al. 2002) and chronic (Zannovich et al. 2002, Liebig et al. 2004) exposure to cold is described as inducing mRNA levels and protein activity. In accord, in our experiment, the levels of UCP1 BAT mRNA were increased in both wild-type and TTR-null mice 1 month (chronic) after exposure to cold, although the increase is relatively mild compared with the changes described after acute exposure.

We expected that in such conditions of increased cellular metabolism as those required in adaptive thermogenesis, the absence of the major thyroid hormone carrier would trigger hypothryoidism and cold intolerance. In fact, hypothryoid rodents (Bianco et al. 2002, Zannovich et al. 2002) and mice depleted of all thyroid hormone receptors (Golozoubova et al. 2004) are cold intolerant. With the present data we clearly show that TTR does not seem to play a buffer or storage role useful for conditions of increased hormone demand. Interestingly, exposure to cold in guinea pigs has been described as decreasing plasma T₄-binding capacity, which was attributed to a decrease in circulating albumin (Yamada et al. 1969). Here we show that, in the cold, TBG mRNA levels are also down-regulated, similarly in TTR-null and wild-type mice. The lower plasma T₄-binding capacity induced by cold would further aggravate impaired response to cold of TTR-null mice, if TTR were important for hormone delivery to tissues, which the present study excludes.

Total thyroidectomy induces marked hypothyroidism and may ultimately result in death. In such extreme situations, it is expected that viability might be prolonged by an increased availability of stored hormone. We chose thyroidectomy to evaluate the importance of TTR-bound T₄ in an acute and extreme condition of thyroid hormone deprivation. Removal of the thyroid gland induces growth arrest (Evans et al. 1964) and both the TTR-null and the wild-type mice presented a marked decrease in body weight gain in comparison with their sham-operated counterparts. Two weeks after ablation of the thyroid, TTR-null and wild-type mice showed similar drastic reductions in T₄ and T₃ plasma and tissue levels, which were not further aggravated 3 weeks after thyroid ablation (data not shown). These observations are in accord with those previously reported in rats. In rats, thyroid hormones tissue levels are strongly reduced upon thyroidectomy and this decrease is not significantly aggravated up to 4 months after thyroidectomy (Obregon et al. 1981). In the present case, it is clear that the TTR-bound T₄ does not delay and/or attenuate the decrease in tissue thyroid hormones, as measured at 2 and 3 weeks after removal of the thyroid gland. TBG mRNA levels, known to be up-regulated in hypothyroidism and to correlate with TBG serum levels (Vranckx et al. 1990), were similarly induced by thyroidectomy in TTR-null and wild-type mice. Therefore, as we have previously shown for TTR-null mice kept in normal housing conditions (Palka et al. 1994), TBG does not compensate for the absence of TTR.

Taken together, adaptation to a moderately or extremely increased need for thyroid hormone does not appear to be influenced by the presence of a TTR-bound T₄ pool. Therefore, TTR does not seem to be important as a reservoir for thyroid hormones in conditions of increased hormone demand. This conclusion raises two major issues, namely, the redundancy of TTR as a plasma hormone carrier, and the importance of further investigating the function of TTR. Redundancy might be expected when other proteins fulfill identical carrier roles, as is the case for albumin and TBG for thyroid hormones. Studies in Nagase analbuminemic rats have excluded a major role for albumin in thyroid hormone homeostasis in normal conditions (Mendel et al. 1989). A single report indicates that analbuminemic rats when fasted and exposed to

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<th>Strain comparison</th>
<th>Thyroid removal effect</th>
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<td>Wild-type</td>
</tr>
<tr>
<td><strong>T₄</strong></td>
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<tr>
<td>Plasma</td>
<td>&lt;0·01</td>
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<tr>
<td>Liver</td>
<td>NS</td>
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<td>Kidney</td>
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<td>Heart</td>
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<td>Cortex</td>
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<td>Cerebellum</td>
<td>NS</td>
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<tr>
<td><strong>T₃</strong></td>
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<tr>
<td>Plasma</td>
<td>NS</td>
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<td>Kidney</td>
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<td>&lt;0·01</td>
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<td>Cortex</td>
<td>NS</td>
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<tr>
<td>Cerebellum</td>
<td>NS</td>
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NS, not statistically significant.
cold survive less than controls (Kawaguchi et al. 1986). However, since albumin is a carrier for many other ligands, including free fatty acids, known to influence survival in stressful situations such as exposure to cold, this is not the appropriate model to study the role of albumin as a thyroid hormone reservoir in conditions such as this. TBG is the major thyroid hormone carrier in man. There are cases of humans with partial or total TBG deficiency, which results in euthyroid hypothyroxinemia (Relfoff 1989, Bartalena 1993). Even though exposure to cold is known to induce alteration in thyroid hormone metabolism in humans (Solter et al. 1989, Sawhney et al. 1995, Do et al. 2004), to the best of our knowledge there are no reports on the ability of these individuals to adapt to cold. Because TTR-null mice are also euthyroid hypothyroxinemic, it is reasonable to believe that humans with TBG deficiency would normally adapt to cold.

It is also possible that in the absence of TTR a compensatory mechanism was developed to substitute TTR in hormone binding. It has been suggested that prostaglandin D2 synthase (L-PGDS) could be such a protein (Beuckmann et al. 1999); however, we have no evidence that L-PGDS is able to bind thyroid hormone in vivo (data not shown). Using electrophoretic and chromatographic approaches we see no other protein replacing TTR in T₄ binding (Palha et al. 2000). It is interesting to note that TTR, also a carrier for retinol, does not seem to impair retinoid metabolism under basal conditions, despite the low retinol found in the serum of TTR-null mice (Wei et al. 1995).

Despite these considerations, it is difficult to accept that TTR, the major protein synthesized by the choroid plexus and whose expression is highly conserved throughout evolution and starts early during embryonic development, plays no more than a biologically redundant role. We therefore consider it important to further investigate the role of TTR. We rather believe that the biological relevance of TTR is more than that initially described in the literature, as a carrier for thyroid hormones. Recently it has been shown that TTR is involved in depression-like behavior and exploratory activity, possibly by modulating noradrenergic transmission in the limbic forebrain (Sousa et al. 2004), and that it acts as a cryptic protease (Liz et al. 2004).

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