The $\Delta337T$ mutation on the TR\textbeta causes alterations in growth, adiposity, and hepatic glucose homeostasis in mice

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Abstract

Mice bearing the genomic mutation $\Delta337T$ on the thyroid hormone receptor $\beta$ (TR\textbeta) gene present the classical signs of resistance to thyroid hormone (TH), with high serum TH and TSH. This mutant TR is unable to bind TH, remains constitutively bound to co-repressors, and has a dominant negative effect on normal TRs. In this study, we show that homozygous (TR$\beta\Delta337T$) mice for this mutation have reduced body weight, length, and body fat content, despite augmented relative food intake and relative increase in serum leptin. TR$\beta\Delta337T$ mice exhibited normal glycemia and were more tolerant to an i.p. glucose load accompanied by reduced insulin secretion. Higher insulin sensitivity was observed after single insulin injection, when the TR$\beta\Delta337T$ mice developed a profound hypoglycemia. Impaired hepatic glucose production was confirmed by the reduction in glucose generation after pyruvate administration. In addition, hepatic glycogen content was lower in homozygous TR$\beta\Delta337T$ mice than in wild type. Collectively, the data suggest that TR$\beta\Delta337T$ mice have deficient hepatic glucose production, by reduced gluconeogenesis and lower glycogen deposits. Analysis of liver gluconeogenic gene expression showed a reduction in the mRNA of phosphoenolpyruvate carboxykinase, a rate-limiting enzyme, and of peroxisome proliferator-activated receptor-$\gamma$ co-activator 1 (PGC1; Wu et al. 2002), or co-repressors (CoR), more importantly the nuclear receptor CoR and the silencing mediator of TR and retinoic acid receptor (Ishizuka & Lazar 2003).

Introduction

Thyroid hormones (TH) exert profound effects on metabolism and growth. Most of TH actions occur through the nuclear TH receptors (TRs), which are ligand-dependent and ligand-independent transcription factors (Yen 2001). TRs are encoded by two distinct genes, $Thr\alpha$ and $Thr\beta$, located on mouse chromosomes 11 and 14 (Thompson et al. 1987, Wood et al. 1991). The use of alternative splicing or transcription initiation sites generates at least three TH-binding isoforms TR$\alpha1$, TR$\beta1$, and TR$\beta2$. These isoforms display a distinct pattern of expression among tissues and during developmental stages (Yen 2001). The molecular mechanism of TH action involves TR binding to TH-responsive elements on the promoter region of target genes (Forman et al. 1992, Shibusawa et al. 2003) and the recruitment of transcriptional co-factors. These co-factors may act as co-activators, including members of the steroid receptor co-activator family (Hong et al. 1997, Ortiga-Carvalho et al. 2005) and the peroxisome proliferator-activated receptor-$\gamma$ co-activator 1 (PGC1; Wu et al. 2002), or co-repressors (CoR), more importantly the nuclear receptor CoR and the silencing mediator of TR and retinoic acid receptor (Ishizuka & Lazar 2003).

Mice bearing TR mutations have been used as a tool to study TH effects in genes regulated by different TR isoforms. Here, we used mice that express the TR$\beta\Delta337T$, which cannot bind TH, being constitutively associated with CoR and acting as a potent dominant negative inhibitor of the WT TR (Safer et al. 1998, Pazos-Moura et al. 2000, Hashimoto et al. 2001). This mouse model reproduces the human syndrome of resistance to TH (Hashimoto et al. 2001). Because of the hypothalamic–pituitary resistance to TH, serum TSH and TH were elevated and tissues present variable degrees of resistance, depending on the type of predominant receptor and on the specific mutation (O’Shea et al. 2006). Previous studies have shown that TR$\beta\Delta337T$ mice presented alterations in cholesterol metabolism (Hashimoto et al. 2006) and were deficient in S cone expression in the retina (Pessôa et al. 2008).
TH effects on growth, body composition, and glucose homeostasis have been described and result from the action of TH on different tissues (Dimitriadis & Raptis 2001, Fukuchi et al. 2002, Bassett et al. 2007); however, the molecular mechanisms and the subtype of TR predominantly involved are not completely understood. Thus, the aim of this study was to investigate the consequences of the impairment of TR signaling on growth, adiposity, and glucose metabolism of mice carrying the Δ337T mutation on TRβ.

Materials and Methods

Ethical approval

This study was approved by the ethics committee of the Health Sciences Center, Federal University of Rio de Janeiro (#IBCCF1002). Animal care and experimental protocols performed are in accordance with those stated by Drummond (2009).

Animals

Mice of different genotypes, wild type (TRβ_{WT/WT}), heterozygous (TRβ_{WT/Δ337T}), and homozygous (TRβ_{Δ337T/Δ337T}) for the TRβΔ337T mutation, were used. Mice were generated as described previously (Hashimoto et al. 2001). All mice were propagated in a mixed 129/C57/BL6 background strain and direct comparisons were made with littermate controls. Animals were generated by heterozygous mating pairs and a total of 79 males were used in this study. The genotyping of tail DNA was performed by PCR using the following primers: 5’ match ATGGGGAAATGG-GAGAG, 5' mismatch ATGGGGAAATGGCAGTAGGAC and 3’ out AGCACACTCACCTGAAGACAT.

Animals were accommodated at controlled room temperature (24±1 °C) and submitted to 12 h light:12 h darkness cycles, lights on at 0700 h.

For all experiments, mice were placed in cages containing four animals and fed standard chow (Bio-Tec, Rio de Janeiro, Brazil) and water made available ad libitum. Body weight (BW) and body length of animals were measured weekly from post-natal week 5 until 24. At the age of 23 weeks, mice were fasted for 12 h (from 1900 to 0700 h) before the test. Animals received an i.p. injection of 0.75 mIU/g BW of human recombinant insulin (Eli Lilly) in PBS. Glycemia was measured before the injection and 15, 30, 60, and 120 min after glucose administration. Blood samples were collected from trunk and serum insulin measured by RIA.

Serum thyroxine, tri-iodothyronine, leptin, and insulin measurements

Specific RIA kits were used to measure serum leptin, insulin (Linco Research, Billerica, MA, USA), total tri-iodothyronine (T3), and total thyroxine (T4; MP Biomedicals, New York, NY, USA) levels. The detection limit and intra-assay variation were 0.5 ng/ml and 3.2% for leptin, 0.1 ng/ml and 5.5% for insulin, 25 ng/dl and 4.6% for total T3, and 1 µg/dl and 1.4% for total T4, respectively. All samples were measured within the same assay.

Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated from simultaneous measurements of serum glucose and insulin in mice under 5 h food restriction.

Glucose tolerance test

Mice were fasted for 12 h (from 1900 to 0700 h) before the test. Animals received an i.p. injection of 2 mg/g BW of (+)-glucose (Merck) in PBS. One group of animals was used to measure the glycemia before the injection and 20, 40, 60, and 120 min after glucose administration, with blood samples obtained from venesection and glucose measured by a glucometer (Optium, MediSense). Another set of animals was killed before glucose injection at 15 and 30 min after glucose administration. Blood samples were collected from trunk and serum insulin measured by RIA.

Insulin sensitivity test

Mice were fasted for 12 h (from 1900 to 0700 h) before the test. Animals received an i.p. injection of 0.75 mIU/g BW of human recombinant insulin (Eli Lilly) in PBS. Glycemia was measured before the injection and 15, 30, 60, 90, and 120 min after glucose administration, with blood samples obtained from venesection and glucose measured by a glucometer (Optium, MediSense).
120 min after insulin administration. Blood samples were obtained by tail vein section and glycemia was measured by a glucometer (Optium, MediSense). The animals used for this test were not used for further analysis presented in this paper. The last two protocols were adapted from Zhou et al. (2004).

Pyruvate challenge test

After 12 h overnight fast, TRβ<sup>WT/WT</sup> and TRβ<sup>337T/337T</sup> mice were injected intraperitoneally with 2 mg/g BW pyruvate (Sigma–Aldrich) dissolved in saline and blood glucose was measured before injection and 15, 30, 60, and 90 min after pyruvate administration. Blood samples were obtained from the tail vein and glycemia was measured by a glucometer (Optium, MediSense).

Liver glycogen content

Liver glycogen content was measured using a previously established protocol (Trevenzoli et al. 2010). Briefly, 250 mg liver samples were homogenized and glycogen was extracted. Glycogen was then hydrolyzed and the resultant glucose was measured using an enzymatic colorimetric method (Glucoc commercial kit, Doles, Goiás, Brazil) and compared to a glucose standard curve.

RNA analysis

Total RNA was extracted from samples by standard methodology (TRizol Reagent; Life technologies, Invitrogen) for analysis of expression of Glh, phosphoenolpyruvate carboxykinase (Pck1), and PGC1α (Ppargc1a) (Trevenzoli 2010). Total RNA was reverse transcribed using 1 μg RNA and the Superscript III (Invitrogen). Real-time PCR was performed on Applied Biosystems 7500 Real-Time PCR System (Life Technologies Corp., Carlsbad, CA, USA) using SYBR Green PCR Master Mix (Applied BioSystems, Rockville, MD, USA). Primers were synthesized by Integrated DNA Technologies and were designed as follows: Glh forward 5’-CAGAGAACGACGACA- TGGAA-3’, Glh reverse 5’-ACTGGATGACGACGCGG-3’, Pck1 forward 5’-ATCTTTGGTGCCGTAGACCT-3’, Pck1 reverse 5’-GCCAGTGCCCAGGTATT-3’, Ppargc1a forward 5’-AGCACCTGACCCATGCGAAC-3’, Ppargc1a reverse 5’-TTTTGTagGACGGGTCTCATCCT-3’, 36B4 primer was used as control as described previously (Machado et al. 2009). Samples were analyzed in duplicate and the cycle parameters were as follows: 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, 60 °C for 30 s, and 70 °C for 45 s. Product purity was confirmed by agarose gel analysis. Changes in mRNA expression were calculated from the cycle threshold, after correcting for 36B4 according to the 2<sup>−ΔΔCt</sup> method (Livak & Schmittgen 2001). Data are expressed as fold induction over WT control group, which was set to 1.

Statistical analysis

Data are reported as mean ± S.E.M. One-way ANOVA was employed when comparisons were made for different genotypes. Two-way ANOVA was employed when comparisons were made for different genotypes along a time course, and analyses were followed by Student–Newman–Keuls multiple comparisons test for assessment of significance (GraphPad Prism; GraphPad Software Inc., La Jolla, CA, USA). Differences were considered to be significant at *P*<0.05.

Results

As expected, TRβ<sup>337T/337T</sup> presented higher levels of serum T₃ compared with the other groups (TRβ<sup>WT/WT</sup>: 103 ± 5, TRβ<sup>WT/337T</sup>: 120 ± 13, and TRβ<sup>337T/337T</sup>: 466 ± 140 ng/dl). BW and length of male mice of different genotypes are shown in Fig. 1. TRβ<sup>337T/337T</sup> male mice displayed lower BW (Fig. 1A) and length (Fig. 1B) compared with TRβ<sup>WT/WT</sup> (*P*<0.05). The differences in BW and length emerged by 14 and 16 weeks of age and persisted thereafter until killing at 26 weeks of age. Heterozygous male mice did not exhibit alterations in length or BW. In female mice, the TRβ<sup>337T</sup> mutation did not affect the BW or length (data not shown). For all the other parameters studied, female mice exhibited the same profile as males, and we here show the results obtained on males, since their alterations were more pronounced.

The reduced BW of TRβ<sup>337T/337T</sup> mice cannot be completely justified by alterations in food intake, since even though in absolute values there was a slight reduction in food intake (data not shown), when corrected for BW, TRβ<sup>337T/337T</sup> mice presented increased chow ingestion compared to 26 weeks of age (Fig. 2A, *P*<0.05). In addition, the reduced apparent growth of TRβ<sup>337T/337T</sup> male mice could not be associated with alterations in pituitary GH mRNA, the expression of which was similar regardless of genotype (Fig. 2B).

TRβ<sup>337T/337T</sup> mice exhibited decreased adiposity as shown by the reduction in abdominal WAT mass (36% less than WT—Fig. 3A) and further confirmed by the lower body fat

Figure 1 Body weight (A) and length (B) of TRβ<sup>WT/WT</sup> (squares), TRβ<sup>WT/337T</sup> (circles), and TRβ<sup>337T/337T</sup> (triangles) male mice from 5 to 24 weeks old. Values are expressed as mean ± S.E.M., *n* = 4–11. *TRβ<sup>WT/WT</sup> versus TRβ<sup>337T/337T</sup>, *P*<0.05, # TRβ<sup>WT/WT</sup> versus TRβ<sup>337T/337T</sup>, *P*<0.01.
content, determined by the carcass method (Fig. 3B, P<0.01). However, there was no significant difference in body protein content among the genotypes (Fig. 3C). Contrary to WAT mass, BAT was enlarged in homozygous-mutant mice (P<0.001, Fig. 3D). Although serum levels of leptin were similar among genotypes, when corrected for body lipid content, a relative increase was evident (Fig. 3E and F, P<0.05).

We next analyzed the metabolic profile of mutant mice concerning glucose homeostasis. After 5 h of fasting, blood glucose levels were similar among different genotypes (Fig. 4A). To maintain normal glycemia, TRβ\(\Delta337T/\Delta337T\) and TRβ\(WT/\Delta337T\) mice needed lower insulin secretion as deduced by serum levels of insulin (Fig. 4B, P<0.05). The HOMA-IR, calculated as the product between serum insulin and blood glucose, was 60% lower in mice carrying the mutation, suggesting that their insulin sensitivity was increased (Fig. 4C, P<0.05). This fact opposes what was seen in one study of patients with RTH where HOMA-IR was 50% elevated in patients carrying different mutations in heterozygosity (Mitchell et al. 2010).

We proceeded with the investigation on insulin sensitivity performing the glucose tolerance test and insulin sensitivity test on 12 h fasted mice (Fig. 5A–C). TRβ\(\Delta337T/\Delta337T\) displayed lower glycemia 20, 40, and 60 min after an i.p. glucose load compared with TRβ\(WT/WT\) (P<0.05). Insulin concentrations measured 15 and 30 min after glucose load revealed that although at 15 min there is no significant differences among groups, homozygous animals failed to increase insulin levels at 30 min (Fig. 5B), indicating that insulin concentrations reduced more rapidly as blood glucose reduced (Fig. 5A and B).

TRβ\(\Delta337T/\Delta337T\) mice were more sensitive to the hypoglycemic effects of insulin and some of them exhibited a severe hypoglycemia (below 20 mg/dl) 60 and 90 min after the i.p. insulin injection and had to be rescued with glucose infusion (Fig. 5C). In case glycemia was below the detection limit (20 mg/dl), mice received an i.p. injection of glucose 2 mg/g BW i.-i. glucose (Merck) in PBS. Heterozygous mice behaved similarly to WT on glucose tolerance test and insulin sensitivity test, leading us to exclude this group from the following evaluations.

In order to have some insight into hepatic glucose production, we evaluated liver glycogen content and the glucose generation after the administration of pyruvate, a precursor for gluconeogenesis. Hepatic glycogen content of TRβ\(\Delta337T/\Delta337T\) was 70% reduced compared with controls (Fig. 6A, P<0.05). TRβ\(WT/WT\) mice displayed an expected increase in blood glucose concentration after pyruvate injection; on the other hand, TRβ\(\Delta337T/\Δ337T\) mice could not reach the same glucose production rate as TRβ\(WT/WT\) mice and exhibited lower blood glucose concentration 30 min after pyruvate injection (Fig. 6B).

Since these results suggested that the gluconeogenic pathway could be impaired in mutant mice, we investigated the hepatic mRNA expression of PEPCK, a key gluconeogenic enzyme, which revealed a reduction in the enzyme mRNA levels in the liver of TRβ\(\Delta337T/\Δ337T\) mice (Fig. 6C, P<0.01) and it was accompanied by a reduction in PEPCK liver mRNA (Fig. 6D, P<0.01), which is an essential transcriptional factor involved in the regulation of gluconeogenic enzymes (Yoon et al. 2001).

![Figure 2](image1.png)

**Figure 2** Daily chow intake corrected for body weight (A) and pituitary Gh mRNA (B) in 26-week-old TRβ\(^{WT}\), TRβ\(^{WT/\Delta337T}\), and TRβ\(^{\Delta337T/\Delta337T}\) male mice. Values are expressed as mean±s.e.m., n=5–8.

![Figure 3](image2.png)

**Figure 3** Abdominal white adipose tissue mass (A), lipidic (B), and proteic (C) body composition, brown adipose tissue (D), serum leptin (E), and serum leptin corrected for body fat mass (F) in 26-week-old TRβ\(^{WT}\), TRβ\(^{WT/\Delta337T}\), and TRβ\(^{\Delta337T/\Delta337T}\) male mice. Values are expressed as mean±s.e.m., n=5–8.
Here, we used mice carrying a human natural mutation in TRβ that does not bind T3 but still binds to DNA and to CoR (Hashimoto et al. 2001). Although the Δ337T mutation in both alleles of the TRβ presents growth impairment, decreased adiposity, and higher insulin sensitivity.

Mutations on the binding domain of TRβ are found in the syndrome of TH resistance leading to high levels of serum TH and to signs and symptoms that are typical of either hyperthyroidism or hypothyroidism. Although mechanisms leading to resistance are not completely clarified and may be mutation specific, it is generally accepted that the resulting phenotype depends on the level of expression of a specific TR isoform in different tissues, and, for tissues where TRα is predominant, signs of hyperthyroidism can be found, the opposite being true for tissues where TRβ is more abundant. Here, we used mice carrying a human natural mutation Δ337T that does not bind T3 but still binds to DNA and to CoR (Hashimoto et al. 2001). Although the Δ337T TRβ has been described to have a potent dominant negative effect over the WT TR, except for 5 h fasting serum insulin, no other significant alterations were observed in heterozygous mice herein studied. Thus, the phenotype of the homozygous mice resulted either from total disruption of TRβ signaling or from the very high levels of TH acting through TRα. Still, another possible mechanism is that a higher dose of the mutant TRβ interferes with the TRα function or with the ability of other transcription factors to regulate gene expression.

TRβΔ337T/Δ337T mice presented a reduced body length and weight despite increased food intake. Both hypo- and hyperthyroidism can lead to growth retardation through different mechanisms (O’Shea et al. 2003, Bassett et al. 2007). TH have potent stimulatory effects over GH and IGF1 production (Miell et al. 1993); however, in this study, despite high serum levels of TH, pituitary GH mRNA was not changed in TRβΔ337T/Δ337T mice. This argues against an impairment of the GH/IGF1 production as a cause of growth retardation in homozygous mice. Thus, it is possible that TRβΔ337T/Δ337T are shorter as a consequence of hyperthyroidism in bone, as seems to be the case for the PV mice, which bear a deletion in TRβ that prevents T3 binding and is a strong dominant negative mutant (O’Shea et al. 2003). For unknown reasons, and contrary to the PV mice that showed growth delay in both genders, TRβΔ337T/Δ337T female mice did not present deficits in growth.

TRβΔ337T/Δ337T mice were also leaner, exhibiting decreased body fat mass in the presence of increased food intake. Previous data demonstrated that TRβPV mice did not present alterations in fat mass (Araki et al. 2009). The lower adiposity found in TRβΔ337T/Δ337T mice may be caused by increased energy expenditure, an expected effect of the high TH levels (Kim 2008), possibly via the TRα. Future studies aiming to evaluate energy expenditure should elucidate this point. In addition, reduced fat mass may result from increased lipolysis, since the elevated TH levels can act through TRα to increase the lipolytic effect of catecholamines (Ribeiro et al. 2001). Previous studies have demonstrated that leptin serum concentrations correlate positively with WAT mass (Licinio et al. 2007), and that TH has the ability to inhibit leptin production (Escobar-Morreale et al. 1997, Cabanelas et al. 2010). However, despite the high serum TH, the mutant mice exhibited increased levels of leptin. This raises the possibility that the inhibitory action of TH on leptin secretion is mediated, in most part, by TRβ. An alternative explanation for the increased serum leptin is related to the high serum production (Miell et al. 2001, Bassett et al. 2007, Araki et al. 2009). The lower adiposity found in TRβΔ337T/Δ337T mice may be caused by increased energy expenditure, an expected effect of the high TH levels (Kim 2008), possibly via the TRα. Future studies aiming to evaluate energy expenditure should elucidate this point. In addition, reduced fat mass may result from increased lipolysis, since the elevated TH levels can act through TRα to increase the lipolytic effect of catecholamines (Ribeiro et al. 2001). Previous studies have demonstrated that leptin serum concentrations correlate positively with WAT mass (Licinio et al. 2007), and that TH has the ability to inhibit leptin production (Escobar-Morreale et al. 1997, Cabanelas et al. 2010). However, despite the high serum TH, the mutant mice exhibited increased levels of leptin. This raises the possibility that the inhibitory action of TH on leptin secretion is mediated, in most part, by TRβ. An alternative explanation for the increased serum leptin is related to the high serum production (Miell et al. 2001, Bassett et al. 2007, Araki et al. 2009). The lower adiposity found in TRβΔ337T/Δ337T mice may be caused by increased energy expenditure, an expected effect of the high TH levels (Kim 2008), possibly via the TRα. Future studies aiming to evaluate energy expenditure should elucidate this point. In addition, reduced fat mass may result from increased lipolysis, since the elevated TH levels can act through TRα to increase the lipolytic effect of catecholamines (Ribeiro et al. 2001). Previous studies have demonstrated that leptin serum concentrations correlate positively with WAT mass (Licinio et al. 2007), and that TH has the ability to inhibit leptin production (Escobar-Morreale et al. 1997, Cabanelas et al. 2010). However, despite the high serum TH, the mutant mice exhibited increased levels of leptin. This raises the possibility that the inhibitory action of TH on leptin secretion is mediated, in most part, by TRβ. An alternative explanation for the increased serum leptin is related to the high serum...
Normal glycemia seems to be maintained in heterozygous and homozygous mice in the presence of lower basal insulinemia after a short period of fasting (5 h), and with normal insulinemia after an overnight fast (12 h). However, TRβA337T/Δ337T mice presented an abrupt decrease in glycemia levels in response to insulin and recovered euglycemic levels faster after glucose load. This increased glucose tolerance was accompanied by lower serum insulin secretion at least in the first 30 min after glucose load (note area under the curve, Fig. 5B). These data indicated that insulin was more efficient in increasing glucose utilization in homoyzgous mice. TH is known to increase insulin-induced muscle glucose uptake and the expression of glucose transporter 4 in WAT and muscle (Weinstein et al. 1994, Torrance et al. 1997, Dimitriadis et al. 2006). Therefore, hyperthyroidism in TRβA337T/Δ337T mice could explain the increased glucose tolerance and insulin sensitivity that, in this case, would be mediated by the TRα. However, experimental hyperthyroidism has also been associated with insulin resistance and with impaired β-cell responsiveness to the stimulatory action of glucose on insulin secretion (Fukuchi et al. 2002, Holness et al. 2008). Therefore, it is possible that the phenotype of the mutant mice results from multiple alterations of the homeostasis of glucose utilization and production caused not only by hyperthyroidism but also by resistance to TH action, as seems to be the case in liver glucose metabolism.

Indeed, TRβA337T/Δ337T mice were unable to recover from the insulin-induced hypoglycemia, which was fatal for some mice. Liver glycogen is the first glucose resource to be utilized in an attempt to return to normoglycemia, and in TRβA337T/Δ337T mice, liver glycogen was reduced by 70%. Hepatic gluconeogenesis, which is necessary for recovery from hypoglycemia, was also impaired in TRβA337T/Δ337T mice, as demonstrated by the reduced conversion of pyruvate to glucose. The mechanism involves the lower liver expression of PEPCK, the rate-limiting enzyme for gluconeogenesis, and PGC1α, an essential transcriptional factor for hepatic gluconeogenesis (Yoon et al. 2001). Despite the known stimulatory effect of TH over these genes’ expression (Weitze et al. 2003, Klieverik et al. 2008), they were not able to respond in the presence of the mutant TRβ. These findings highlight the role of TRβ in mediating TH effects on hepatic glucose production. The central control of hepatic glucose production is also subject to regulation by TH as was demonstrated in hyperthyroid rats that showed an increase in glucose production and resistance to insulin (Klieverik et al. 2008). When infused centrally, TH produced and increased in plasma glucose, an effect mediated by sympathetic nerve activity in the liver (Klieverik et al. 2009).

The great majority of patients with the syndrome of TH resistance are heterozygous for the TRβ mutation. The rare cases of homozygous presentation were very severe (Ono et al. 1991), and cardiovascular disorders have been associated with lethality. However, in view of our data, and in spite of
species differences, severe hypoglycemia due to impaired hepatic glucose production may be investigated as another contributing factor.

In conclusion, the Δ337T TRβ mutation, associated with the syndrome of resistance to TH, resulted in reduced BW and length in male mice and metabolic alterations in both genders. Reduced fat accumulation was accompanied by an increase in food intake despite relatively higher leptin. More importantly, TRβΔ337T/Δ337T mice were more tolerant to glucose but more sensitive to the hypoglycemic effect of insulin due in part to an important deficit in hepatic glucose production.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

The conception and design of this study was conducted by L A S, T M O-C, and C C P-M. F E W was responsible for the generation of the mutant mouse. Experiments were performed by L A S, D A S, L C F , A C, and P C L. The conception and design of this study was conducted by L A S, T M O-C, and C C P-M. F E W was responsible for the generation of the mutant mouse. Experiments were performed by L A S, D A S, L C F , A C, and P C L. All authors contributed to the analysis and interpretation of data as well as writing, revising, and final approval of the version to be published. The study was conducted at the Instituto de Biofísica Carlos Chagas Filho, Federal University of Rio de Janeiro, Brazil.

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