



The effects of 3,5-diiodothyronine on energy balance

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INTRODUCTION

Thyroid hormones (THs) have been known to affect energy metabolism (calorigenic effect) for over a century (Magnus-Levy, 1895; Thompson et al., 1929). In 1985 Magnus-Levy observed that patients with mixedema exhibited an abnormal low oxygen consumption when compared to normal individuals and that unusually higher amount of oxygen was consumed by hyperthyroid patients. 3,3',5-triiodo-L-thyronine (T_3) is the active form of THs and it is a major regulator of growth and development and of cellular and tissue metabolism (both intermediate and energy metabolism) throughout the body. Metabolic actions include regulation of: basal metabolic rate in homeotherms, synthesis of mitochondrial respiratory enzymes and membranes, oxidative phosphorylation and energy transduction, movement of water and Na^+ ions across cell membranes; calcium and phosphorus metabolism, lipids synthesis and storage, catabolism of fatty acids, cholesterol, carbohydrate; and nitrogen (urea, creatine) metabolism; growth and developmental actions include actions on: rate of postnatal growth of many mammalian and avian tissue, maturation of fetal brain and bone, amphibian larval metamorphosis, and molting in birds. It is now recognized that T_3 affects gene expression in target tissues/cells by binding to its cognate nuclear receptors (TR) which are ligand-inducible transcription factors. Two TR genes α and β encode four T_3 -binding receptor isoforms (α_1 , β_1 , β_2 , and β_3). The transcriptional activity of TRs is regulated at multiple levels. Besides being regulated by T_3 , transcriptional activity is also regulated: (i) by the type of thyroid hormone response

elements located on the promoters of T_3 target genes, (ii) by the developmental- and tissue-dependent expression of TR isoforms, and (iii) by a host of nuclear coregulatory proteins (corepressors and coactivators). These nuclear proteins modulate the transcription activity of TRs in a T_3 -dependent manner. In the absence of T_3 , corepressors act to repress the basal transcriptional activity, whereas in the presence of T_3 , coactivators act to activate transcription. The activities regulated via the previous described mechanisms are described as "genomic actions." However, between the mid-1980's and the beginning of the 1990's it became evident that some TH effects are non-genomic in origin. Indeed, high-affinity binding sites for thyroid hormones have for many years been recognized on the plasma membrane and other cellular sites such as mitochondria and cytoplasm (for review see Cheng et al., 2010). Recently, a structural protein of the plasma membrane, integrin $\alpha v \beta 3$, has been shown to contain a binding domain for iodothyronines that is an initiation site for hormone-directed complex cellular events, such as cell division and angiogenesis (Bergh et al., 2005) and this qualifies the binding site for characterization as a receptor. Examples of non-genomic action of thyroid hormones are activation of: membrane Ca^{2+} -ATPase activity, 2-Deoxyglucose transport, Na^+ , K-ATPase activity, Na^+ current in myocardiocytes, Na^+ current in sensory neuron, Na^+/H^+ exchanger, cancer cell proliferation, angiogenesis (for review see Cheng et al., 2010). In addition to this, it is now recognized that other iodothyronines or THs analogs/derivatives are able to exert relevant biological actions (for recent review, see Moreno et al., 2008; Senese

et al., 2014; Zucchi et al., 2014). This article is particularly intended to describe the effects of the 3,5 diiodo-L-thyronine (T_2) on energy balance (Moreno et al., 1997; Goglia, 2005).

3,5-DIODO-L-THYRONINE (T_2)

T_2 , a naturally occurring diiodothyronine, is a product of a currently unknown enzymatic process most probably utilizing T_3 as its precursor (Moreno et al., 2002). Some years ago surprising results were published showing that (among a lot of iodothyronines tested) T_2 , at a very low concentration (pM), induced a rapid stimulation of oxygen consumption in perfused livers isolated from hypothyroid rats. In the same study, it was shown that T_3 showed a similar effect but this effect was largely abolished by the addition of an inhibitor of D1 deiodinase, while the effect of T_2 was not. Moreover, T_2 exerted its effect more rapidly than T_3 (Horst et al., 1989). Stimulated by that report and another study showing an interaction of a diiodothyronine with mitochondria (Goglia et al., 1981) some laboratories started to investigate more deeply on possible specific biological actions of T_2 . Initially, energy metabolism was the major area of interest. Indeed, several reports from various laboratories showed that acute or chronic administration of T_2 to rats resulted in significant changes in energy metabolism. When either T_3 or T_2 were acutely injected to hypothyroid rats, T_2 had a more rapid effect on resting metabolic rate than T_3 (Lanni et al., 1996). The experimental design used in this study was basically the same as that employed by Tata in the early 1960's (Tata et al., 1962; Tata, 1963) and the only difference was that in the study of Lanni

et al. hypothyroidism was achieved by the simultaneous administration of propylthiouracil (PTU) and iopanoic acid (IOP). This treatment produces animals with severe hypothyroidism and at the same time, with a powerful inhibition of all three types of deiodinase enzymes. In such conditions the effects of T_2 were evident as soon as 1 h after its injection reaching the maximal value after 24 h, while that of T_3 became evident only after 24 h reaching the maximal value after 72 h (these effects of T_3 were overlapping to those obtained by Tata, 1963). Moreover, while the effect of T_3 was inhibited by an inhibitor of transcription such as actinomycin D (as shown also by Tata, 1963), the effect of T_2 was not (Lanni et al., 1996; Moreno et al., 1997) (see **Figure 1**).

Following these results studies continued to try to clarify the mechanisms underlying the previous described results. In light of the effects of T_2 on energy metabolism, the mitochondria became the obvious candidates to study such mechanisms. In this context, some years ago, by top-down elasticity analysis, it was showed a stimulation of the activity of both cytochrome c-oxidizers and the cytochrome c-reducers components of the respiratory chain, 1 h after the injection of 3,5- T_2 (Lombardi et al., 1998). These data indicate a possible direct interaction of T_2 with some components of the respiratory chain. Indeed, this hypothesis was

in agreement with previous results showing a direct stimulation of the enzyme cytochrome oxidase (COX) activity isolated from bovine heart (Goglia et al., 1994). Arnold and Kadenbach (1997) showed that (in addition to the mitochondrial membrane potential, the substrate pressure in the respiratory chain and the oxygen concentration) the respiration of animal cells is also controlled by the matrix ATP/ADP ratio, via an interaction of nucleotides with COX. In fact, ATP produces an allosteric inhibition of the COX activity. In a further investigation, Arnold et al. (Arnold et al., 1998) showed that 3,5- T_2 specifically binds to subunit Va of the COX complex and completely abolishes the allosteric inhibition of respiration induced by ATP. Subunit Va of the COX complex is therefore a mitochondrial site through which T_2 may directly affect mitochondrial activities. In addition to activating mitochondrial substrate oxidation, 3,5- T_2 also stimulates skeletal mitochondrial uncoupling in a very rapid manner (Lombardi et al., 2007, 2009). By discriminating between proton-leak and redox-slip processes, an increased mitochondrial proton conductance has been addressed as the “pathway” underlying the effect of T_2 on mitochondrial uncoupling. Thus, activation of COX complex above described associated to changes in the efficiency of the skeletal muscle mitochondrial energy-transduction apparatus, may

explain, at least in part, the rapid effect of T_2 on metabolic rate.

The stimulatory effects exerted by T_2 on RMR prompted my group to investigate on a possible effect of this iodothyronine in counteracting overweight and lipid accumulation without deleterious side effects in particular on heart and skeletal muscle such as those showed by T_3 when tested as slimming agent. To test this idea, we administered T_2 for 30 days to rats on a high fat diet (HFD) and then we measured the adipose tissue mass, the body weight gain, the liver adiposity, the liver fatty acid oxidation rate, and the serum levels of triglyceride, fatty acids, and cholesterol. In this study we also looked at a possible effect of T_2 on HPT axis (Lanni et al., 2005). The results showed that, except a slight decrease (–20%) in T_4 serum level, no variation in the Hypothalamus-Pituitary-Thyroid axis (HPT) was evident measured by the “TRH-test.” In this study rats treated with T_2 showed lower body weight, a higher liver fatty acid oxidation rate, less fat mass, an almost complete disappearance of fat from the liver, and significant reductions in the serum triglyceride and cholesterol levels. Recently, most of these results have been confirmed also in animals with a standard laboratory diet and with a prolonged time of treatment (Padron et al., 2014). In addition, several studies from both our and others laboratories showed relevant biological effects of T_2 and some of them reported beneficial effects of T_2 , among others:

Moreno et al. (2011) and de Lange et al. (2011) showed that T_2 prevented high-fat-diet-induced insulin resistance in rat whose action involved activation of sirtuin 1 (SIRT1).

Markova et al. (2013) showed an antidepressant-like effect of T_2 in rats after a bolus administration of T_2 at the doses 75 and 150 $\mu\text{g}/100\text{ g b.w.}$

Shang et al. (2013) showed that T_2 was a protective agent against renal damage in diabetic nephropathy in streptozotocin-induced diabetic rats, confirming the involvement of sirtuin 1 (SIRT1).

These effects were observed in absence of deleterious side effects. However, some

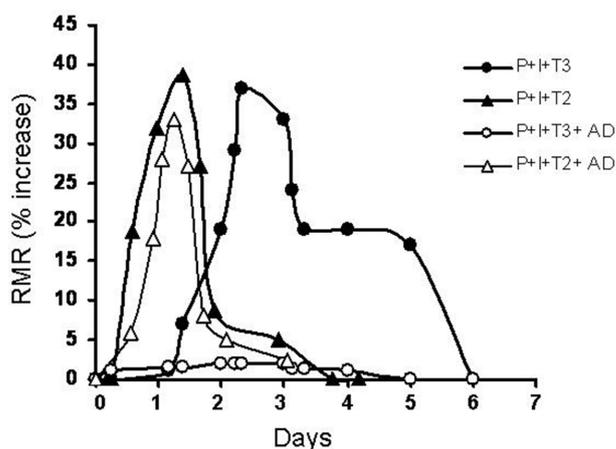


FIGURE 1 | Time course of variation in the resting metabolic rate of hypothyroid rats following administration of a single dose of iodothyronines (25 $\mu\text{g}/100\text{ g BW}$ for both T_2 and T_3) with or without a concomitant administration of actinomycin D (8 $\mu\text{g}/100\text{ g BW}$) (AD).

Hypothyroidism was induced by combined treatment with PTU and IOP (P + I). Resting metabolic rate (RMR) is reported as % increase [vs. time 0 (immediately before the injection)].

studies have shown some deleterious effect at cardiac level and on HPT axis (Goldberg et al., 2012; Jonas et al., 2014) but in these studies unusual very high doses of T₂ were studied and unspecific interaction of T₂ with nuclear TRs may have occurred.

Further details about the actions of T₂ can be found in some reviews (Goglia, 2005; Coppola et al., 2014; Senese et al., 2014).

Of Note, the understanding of the physiological and pathophysiological role of T₂ would benefit from development and standardization of new methods for analytical measurement of T₂ and other TH derivatives. Actually, measurements of T₂ in human tissue and sera have been so far taken using immunoassays (Kirkegaard et al., 1981; Nishikawa et al., 1981). However, the lack of labeled iodothyronines with high specific activity as well as of specific antibodies has represented an important limitation to the application of such approach. Promising developments in this field have recently emerged from the optimization of a new competitive chemiluminescence immunoassay (CLIA) based on the use of one selected mouse monoclonal anti-T₂ antibody with very low cross-reactivity to structurally related THs and thyronamines (Lehmpful et al., 2014). Mass spectrometry techniques have also drawn attention to the analyses of iodothyronines (Köhrle et al., 2013), however, intrinsic instrumental limits still restrain the application of such approaches as routine tools.

CONCLUSION AND PERSPECTIVES

The data reported and discussed in this article have generated a large interest in the possibility of identifying analogs/derivatives of thyroid hormone that may prove effective as therapeutic agents to counteract some major diseases that are growing in importance worldwide. But, before this perspective can be realized further studies are needed to elucidate the cellular/molecular mechanisms of action of these agents and, in addition, a possible therapeutic use of these agents need deep investigations on possible deleterious side effects especially when administered for a long time. As for T₂ it remains to be established whether it has a physiological role or not and in an affirmative case it remains unclear what are

the possible physiological roles that differentiate the actions of T₂ and T₃. Another aspect is related to the problem of possible TR-mediated genomic effect of T₂. It has been recently proven that T₂ is a specific ligand for a long isoform of TR β in tilapia (Mendoza et al., 2013) affecting the growing processes in this specie. T₃ and T₂ both participate in the growth process, however their effects are mediated by different, specific TR β 1 isoforms (STR β 1 and LTR β 1 respectively). However, no data are present at the moment showing that a such binding can affect energy metabolism in higher species and further studies are needed to verify this possibility. Further studies may also be useful to try to develop new concepts that could help toward a better understanding of some of the effects of thyroid hormones and those of their analogs/derivatives.

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