Thyroid hormone receptor β -specific agonist GC-1 increases energy expenditure and prevents fat-mass accumulation in rats

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Abstract

It is well known that thyroid hormone affects body composition; however, the effect of the thyroid hormone receptor β (TR β)-selective thyromimetic GC-1 on this biological feature had not been demonstrated. In the current study, we compared the effects of a 6-week treatment with triiodothyronine (T3; daily injections of 3 or 6 μ g/100 g body weight) or GC-1 (equimolar doses) on different metabolic parameters in adult female rats. Whereas all animals gained weight (17–25 g) in a way not basically affected by T3 or GC-1 treatment, only T3 treatment selectively increased food intake (50–70%). Oxygen consumption was significantly and equally increased (50–70%) by T3 and GC-1. Analysis of body composition by dual-energy X-ray

absorptiometry (DEXA) revealed that, whereas control animals gained about 80% of fat mass, T3- or GC-1-treated animals lost 70–90 and $\sim$ 20% respectively. Direct analysis of the carcass showed that T3 treatment promoted a 14–74% decrease in fat content but GC-1 treatment promoted only a 15–23% reduction. The gain in lean mass by DEXA and the carcass protein content were not affected by T3 or GC-1 treatment. However, the mass of individual skeletal muscles was negatively affected by T3 but only barely by GC-1. These findings highlight the potential use of GC-1 for the treatment of obesity and the metabolic syndrome.

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Introduction

Thyroid hormone controls gene expression by interacting with nuclear receptors (TRs), which are hormone-inducible transcriptional factors (Lazar 1993). Two genes encode TRs, TR α and TR β (Freedman 1992). At least two isoforms of TR α and TR β have been identified and are the result of alternative splicing. TR α 1, TR β 1 and TR β 2 isoforms bind T3, while TR α 2 does not and functions, at least *in vitro*, as a TR α 1 and TR β 1 antagonist (Katz & Lazar 1993). Activation of TRs causes a multitude of physiological effects that range from actions on the embryonic development to actions on the maintenance of energy homeostasis in adults (Motomura & Brent 1998).

It is fascinating that an excess of thyroid hormone can result in some therapeutically desirable effects, such as increased metabolic rate, lipolysis, cholesterol lowering, improvement of heart contractility and thyroid-stimulating hormone (TSH) suppression, which is beneficial in patients with thyroid cancer (Motomura & Brent 1998). At the same time, systemic

thyrotoxicosis also results in undesirable effects, including tachycardia, arrhythmia, muscle wasting, nervousness, fatigue and loss of bone mass (Motomura & Brent 1998). A series of studies in mice with inactivation or mutation of different TR isoforms (Fowler *et al.* 1996, Fraichard *et al.* 1997, Johansson *et al.* 1998, Wikstrom *et al.* 1998, Gothe *et al.* 1999, Gauthier *et al.* 2001, Flamant *et al.* 2002), as well as studies in patients with the syndrome of resistance to thyroid hormone (characterised by mutations in TR β ; Refetoff 2003, Cheng 2005), suggests that TR isoforms selectively mediate tissue-specific thyroid hormone responses. It is a general consensus that TR α mediates the effects of thyroid hormone on the heart, while TR β mediates its effects on plasma cholesterol and TSH secretion, some of the desirable effects of systemic thyrotoxicosis (Baxter *et al.* 2004). Therefore, the development of T3 analogues with preferred TR β binding action could be a possible strategy of achieving desirable effects of T3 and bypassing the undesirable effects.

GC-1 is a synthetic analogue of thyroid hormone that is relatively selective for both binding and activation functions

of TR β 1 over TR α 1 (Chiellini *et al.* 1998). GC-1 has several structural differences with respect to the natural hormone T3, including replacement of the three iodine residues with methyl and isopropyl groups, of the biaryl ether linkage with a methylene linkage and of the amino acid side chain with an oxyacetic acid side chain (Chiellini *et al.* 1998). GC-1 binds TR β 1 with the same affinity as it does T3 but binds TR α 1 with an affinity about ten times lower than that for T3. It is interesting that GC-1 presents selective actions *in vitro* (Chiellini *et al.* 1998) and *in vivo* (Trost *et al.* 2000, Ribeiro *et al.* 2001). The differential effects of GC-1 vs T3 on the thermogenesis by brown adipose tissue (Ribeiro *et al.* 2001), tadpole metamorphosis (Furrow *et al.* 2004) and the development of the bone and central nervous system (Morte *et al.* 2002, 2004, Manzano *et al.* 2003, Freitas *et al.* 2005) may be the result of GC-1 selectivity for TR β . On the other hand, the selective effects of GC-1 may also be related to the body distribution of TR isoforms. In agreement with studies in which the TR β gene has been disrupted (Johansson *et al.* 1999), GC-1 has almost no effects on the heart, which expresses mainly TR α 1, but lowers serum levels of cholesterol and triglycerides, in agreement with a predominant expression of TR β 1 in the liver (Trost *et al.* 2000). Other studies also suggest that the selective actions of GC-1 might also be explained by differential tissue uptake since it presents clear tissue-specific accumulation (Trost *et al.* 2000, Baxter *et al.* 2004). It has been shown, for example, that GC-1 accumulates selectively in the liver as compared with the heart. The tissue/plasma ratio was similar for GC-1 and T3 in the liver but was 30 times lower in the heart (Trost *et al.* 2000).

It is well known that thyrotoxicosis affects body composition, reducing fat mass and lean mass (Lonn *et al.* 1998, Riis *et al.* 2005). In primates, treatment with GC-1 has been shown to increase oxygen consumption and to reduce body weight (Grover *et al.* 2004), but effects on body composition had not been determined yet. Here, we show that treatment with GC-1 increased the metabolic rate, had no effect on food intake and decreased fat mass while sparing the lean mass of rats. These data illustrate the potential of GC-1 as an agent for the treatment of metabolic disorders, including obesity.

Materials and Methods

Animals and drugs

All experimental procedures were performed in accordance with the guidelines of the Standing Committee on Animal Research of the University of Sao Paulo. Female Wistar rats were obtained from our breeding colony and maintained under controlled conditions of light and temperature (12 h darkness:12 h light cycle at 25 °C). All animals were kept in plastic cages, four per cage, and had free access to food (rat chow containing 1.4% Pi, 0.7% Ca and 4.5 IU/g vitamin D) and water. At the age of 100 days and a weight of 210–220 g, the animals were randomly divided into five groups ($n = 8$ per

group): (i) control, treated with saline; (ii) 10 $\times$ T3, treated with 3 μ g T3/100 g body weight (BW) per day, which is equivalent to ten times the physiological dose of T3 (Bianco & Silva 1987); (iii) 20 $\times$ T3, treated with double of the previous T3 dosage (6 μ g T3/100 g body weight (BW) per day); (iv) 10 $\times$ GC-1, treated with 1.5 μ g GC-1/100 g BW per day and (v) 20 $\times$ GC-1, treated with 3 μ g GC-1/100 g BW per day. The latter two groups were treated with GC-1 in equimolar doses of 10 $\times$ T3 and 20 $\times$ T3 respectively. The equimolar doses of GC-1 were calculated from the molecular mass of T3 (mol mass = 651) and GC-1 (mol mass = 328.4). T3 (Sigma Chemicals) was dissolved in 40 mM NaOH, and GC-1 was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 1 mg/ml; either T3 or GC-1 were then diluted in saline and administered i.p. every day for 6 weeks. BW was measured thrice a week (on Mondays, Wednesdays and Fridays). Body length (nose to base of the tail) was determined at the end of the experimental period.

Serum parameters

At the end of the experimental period, the animals were killed by decapitation and the blood of the trunk was collected. The serum was separated by centrifugation and immediately frozen. Total thyroxine (T4) and T3 serum levels were measured by commercial RIA kits (RIA-gnost T4 and RIA-gnost T3, CIS Bio international, France). For the T4 and T3 assays, standard curves were constructed in our laboratory with a pool of charcoal-stripped rat serum. To determine whether GC-1 could cross-react in the T4 and T3 RIAs, we measured serum levels of T4 and T3 in hypothyroid rats treated with different doses of GC-1. Serum T4 was undetectable in all animals, and no differences were seen in serum levels of T3 between the GC-1-treated animals and the hypothyroid-untreated animals, indicating that T4 and T3 RIAs cannot detect GC-1.

Food intake

Once a week (weeks 1–6), the animals were individually maintained in metabolic cages for 24 h for determination of food intake. The rat chow (60–70 g) was weighed before (initial weight) and after (final weight) placing the animals in the metabolic cages for 24 h. Food intake was calculated by the difference between the final and initial weight of the chow (food intake = initial weight – final weight). A basal measurement was done 1 week before the initiation of T3 or GC-1 treatment (week 0).

Determination of body composition by dual-energy X-ray absorptiometry (DEXA)

Lean mass (LM) and fat mass (FM) were measured by dual-energy X-ray absorptiometry (DEXA) using the pDEXA

Sabre Bone Densitometer and the pDEXA Sabre Software version 3.9.4 (Norland Medical Systems, Fort Atkinson, WI, USA), both specially designed for small animals. The research mode scan option was used for the measurements. Pixel spacing for the scan was set to 0.5×1.0 mm; the scan width to 10.5 cm; the scan length to 11.5 cm and the scan speed to 8 mm/s. Since the scan window of the bone densitometer was not large enough to allow a total body scan of the rats used in this study, the scans were performed from the first lumbar vertebra to the hind limbs. This region was named hind body (HB) in the present study. For the scans, the animals were anaesthetised with a cocktail of ketamine and xylazine (30 and 10 mg/kg BW i.p. respectively) and scanned in the prone position. The animals were scanned before (basal scan) and after (final scan) 6 weeks of treatment. Δ LM and Δ FM were calculated, for each animal, by the difference between both scans (final scan–basal scan). For the scan analysis, the bone mineral density (BMD) histogram averaging width (BHAW) was set to 0.01 g/cm^2 for all scans. This parameter can be considered as a variable window that ‘refines’ the distribution of the scan data and allows the definition of the threshold for bone and soft tissues (LM and FM). To establish the appropriated BHAW to this study, we analysed scans of six rats. Two regions of interest (ROIs) were used: HB and soft tissue (ST), a ROI of 1 cm^2 that included exclusively soft tissue. This ROI was placed 0.5 cm to the rear of the left femur and 0.5 cm to the left of the pelvis. BMD, LM and FM of HB and ST were measured using various BHAWs, ranging from 0.005 to 0.2 g/cm^2 . The values for each parameter were plotted and analysed. BMD and LM values increased and FM values decreased from the BHAW of 0.005 – 0.01 g/cm^2 . From the BHAW of 0.01 – 0.2 g/cm^2 , a plateau was established for each parameter. Thus, 0.01 g/cm^2 was the BHAW of choice since it determined the first point in the plateau and allowed the greatest discrimination among BMD, FM and LM for all scans. The precision *in vivo* was evaluated by calculating the coefficient of variation ($\text{CV} = 100 \times \text{s.d./mean}$) of six repeated measurements of a 2-month-old female rat weighing 207 g. The animal was repositioned after each scan. The CV of LM and FM of the HB were 1.7 and 1.9% respectively. The precision *in vitro* was also expressed as CV and calculated by measuring the BMD of a phantom, with a nominal density of 0.929 g/cm^2 . This CV was 0.8% throughout the experiment. The performance of the system was assessed and maintained by the quality assurance test (QA test), which includes scanner calibration and phantom scanning. The QA test was carried out on each day that scans were to be performed.

Body composition by carcass analysis

Carcasses were thawed overnight at 4°C , reweighed, cut in small pieces and then thoroughly homogenised with distilled water (the volume of water equals double the weight of the carcasses) in a blender (Kinematica AG., Lucerne,

Switzerland). Aliquots of homogenate were used for measurement of water, protein and fat content. The water content was determined according to the method of Bertin *et al.* (1998). Briefly, two aliquots of homogenate per animal were weighed before and after drying for 18 h in an oven at 103°C . Fat content was determined from triplicate aliquots of homogenate per animal according to Folch *et al.* (1957). Briefly, lipid was extracted from 0.3 g aliquots of homogenate with a 2:1 chloroform:methanol solution. The lipid-containing chloroform layer was separated and dried to a constant weight. The protein content was determined by the biuret method, as described by Brooks *et al.* (1995).

Weight of fat pads, skeletal muscles and heart

Immediately after animals were killed, the retroperitoneal fat pad, the heart and the quadriceps, gastrocnemius, soleus and extensor digitorum longus (EDL) were carefully dissected out and weighed (wet weight). The samples of the skeletal muscles were then transferred to an incubator at 60°C for 48 h and weighed again (dry weight). The water content of each sample was determined by the difference between the wet and dry weights (water content = wet weight – dry weight). All weights were expressed in mg/g BW.

Oxygen consumption

Resting oxygen consumption (VO_2) was measured in conscious rats in an open-circuit respirometer system (S-3A/1, Ametek, Pittsburgh, PA, USA) as previously described (Withers 1977) at the end of the experimental period. All measurements were carried out at room temperature ($>25^\circ\text{C}$) over a period of 1 h during the morning (0900–1200 h). Animals were studied while they were in the fed condition and were maintained under normal experimental conditions until immediately before the measurements were taken. Online data were collected and analysed with a computer system running on DataCan V software (Sable Systems, Salt Lake City, UT, USA). Results were corrected for environmental temperature and atmospheric pressure and expressed in terms of microlitres of O_2 per min/g BW.

Statistical analysis

One-way ANOVA was used to compare more than two groups and was always followed by the Student–Newman–Keuls test to detect differences between groups. For all tests, $P < 0.05$ was considered statistically significant. All results are expressed as the mean $\pm$ S.E.M. For statistical analysis, we used the GraphPad InStat Software (GraphPad Software, San Diego, CA, USA).

Table 1 Serum levels (ng/ml) of T3 and T4. Values are the means $\pm$ S.E.M. ($n=8$ for all groups)

	T3	T4
Group		
Control	1.02 $\pm$ 0.2	48.0 $\pm$ 2.7
10 $\times$ T3	>2	30.2 $\pm$ 1.2*
20 $\times$ T3	>2	31.9 $\pm$ 3*
10 $\times$ GC-1	0.80 $\pm$ 0.07	46.7 $\pm$ 1.3
20 $\times$ GC-1	0.84 $\pm$ 0.09	32.9 $\pm$ 2.9*

Significance among groups was determined by ANOVA followed by Student–Newman–Keuls test. * $P<0.001$ vs control.

Results

Serum levels of T3 and T4

As expected, serum levels of T3 were significantly higher in the 10 $\times$ T3- and 20 $\times$ T3-treated groups than in the controls (Table 1). Serum levels of T3 were not affected by treatment with GC-1. On the other hand, serum levels of T4 were lower in the 10 $\times$ T3-, 20 $\times$ T3- and 20 $\times$ GC-1-treated animals than in the controls.

Effects of T3 and GC-1 on body weight and food intake

Body weight was barely affected by T3 and was not affected by GC-1 (Fig. 1A and B). Both 10 $\times$ T3 and 20 $\times$ T3 treatments reduced body weight by approximately 6 and 10% when compared with controls ($P<0.05$ for both) respectively, from day 6 to day 10 of treatment. Rats treated with 20 $\times$ T3 also presented with a lower body weight than control rats (-7.4% , $P<0.05$) from day 24 to day 27 of treatment, but by the end of the treatment period, all animals were the same body weight. The body length was not affected by T3 or GC-1.

Treatments with 10 $\times$ T3 and 20 $\times$ T3 increased food intake by 50 and 72% ($P<0.001$ for both vs controls) respectively, in week 2 of treatment, while treatment with GC-1 had no effect on food intake (Fig. 1C). Food intake in the T3-treated animals remained $\approx 63\%$ greater than that in controls until the end of the treatment period.

Effects of T3 and GC-1 on oxygen consumption

At the end of the experimental period, resting oxygen consumption (VO_2) was significantly and equally increased by treatment with T3 or GC-1 by about 46–88% when compared with that of controls (Fig. 2).

Effects of T3 and GC-1 on fat mass and fat content

Analysis of body composition by DEXA showed that treatment with T3 or GC-1 negatively affected ΔFM (Fig. 3A). While control rats presented an increase of

Figure 1 Effects of T3 and GC-1 on body weight and food intake. (A) Body-weight response to treatment with T3. (B) Body-weight response to treatment with GC-1. Body weight was measured thrice a week in control rats and rats treated for 6 weeks with T3 or GC-1. (C) Food intake was determined weekly in control rats and rats treated for 6 weeks with T3 or GC-1. All values (A–C) are expressed as the mean $\pm$ S.E.M. ($n=8$ per group). * $P<0.05$ and † $P<0.001$, control vs 10 $\times$ T3 and 20 $\times$ T3 and ‡ $P<0.05$, control vs 20 $\times$ T3 by Student–Newman–Keuls test.

Figure 2 Effects of T3 and GC-1 on oxygen consumption. Resting oxygen consumption was measured at the end of the experimental period in control rats and rats treated for 6 weeks with T3 or GC-1. All values are expressed as the mean $\pm$ S.E.M. ($n=5$ per group). * $P<0.01$ and † $P<0.001$ vs control by Student–Newman–Keuls test.

approximately 83% (6.5 g) in fat mass, 10×T3- and 20×T3-treated rats lost approximately 74% (4.2 g) and 91% (5.9 g) respectively, of their fat mass. Treatment with 10×GC-1 resulted in a gain of fat mass of only 21% (1.7 g), and treatment with 20×GC-1 induced a decrease in fat mass of 20% (1.3 g).

The determination of fat content by analysis of the carcass showed that 10×T3, 20×T3, 10×GC-1 and 20×GC-1 significantly reduced the fat content by 14, 74, 15 and 23% respectively, when compared with controls (Fig. 3B). Treatment with 10×T3, 20×T3 and 20×GC-1 significantly reduced the mass of the retroperitoneal fat pad by 68, 73 and 48% respectively (Fig. 3C).

Effects of T3 and GC-1 on lean mass, protein content, skeletal muscle mass and heart mass

Treatment with T3 or GC-1 did not affect Δ LM, as determined by DEXA, and the protein content, as determined by carcass analysis. Nevertheless, skeletal muscle mass was negatively affected by T3 (Table 2). The wet and dry masses of the quadriceps, gastrocnemius and soleus of the T3-treated animals were significantly lower (13–36%) than those of the control animals, while these parameters were not affected in the EDL muscle. Treatment with 20×GC-1 significantly reduced wet and dry masses of the quadriceps by 16 and 24% respectively. None of the other skeletal muscles were affected by GC-1. The wet mass of the quadriceps increased 15% ($P<0.01$ vs control) after treatment with 10×GC-1, which can be explained by an increase in its water content (19% vs control, $P<0.001$). As expected, treatment with 10×T3 or 20×T3 resulted in cardiac hypertrophy, characterised by an increase in the dry mass of the heart (33 and 46% vs control respectively), while GC-1 treatment had no effect.

Figure 3 Effects of T3 and GC-1 on change in fat mass, carcass fat content and retroperitoneal fat pad. All parameters were determined in control rats and rats treated for 6 weeks with T3 or GC-1. (A) Δ Fat mass (final fat mass–basal fat mass) was determined *in vivo* by DEXA. (B) Carcass fat content. (C) Retroperitoneal fat pad. All values are expressed as the mean $\pm$ S.E.M. ($n=8$ per group). * $P<0.05$, † $P<0.01$ and ‡ $P<0.001$ vs control by Student–Newman–Keuls test.

Discussion

The present study shows that treatment with GC-1 increases VO_2 (Fig. 2), a finding corroborated by a previous report (Grover *et al.* 2004). It is interesting however that 10×GC-1 and 20×GC-1 increased VO_2 by 53 and 90% respectively, whereas Grover *et al.* (2004) observed only modest (5–10%) increases in VO_2 with doses of GC-1 in this range. Such a discrepancy might be explained by the much longer duration of GC-1 treatment in the current investigation (1 vs 6 weeks).

Table 2 Effects of T3 and GC-1 on skeletal mass and heart mass

	Control	10×T3	20×T3	10×GC-1	20×GC-1
Muscle					
Quadriceps					
Wet mass	7.97±0.28	6.38±0.33 [†]	6.90±0.16*	9.19±0.36 [†]	6.62±0.28 [†]
Dry mass	2.27±0.11	1.78±0.10 [†]	1.96±0.07*	2.44±0.14	1.73±0.08 [†]
H ₂ O content	5.69±0.19	4.60±0.23 [†]	4.94±0.10*	6.75±0.24 [‡]	4.90±0.21*
Gastrocnemius					
Wet mass	4.66±0.14	3.00±0.19 [†]	3.95±0.28	4.71±0.22	4.55±0.15
Dry mass	1.31±0.06	0.83±0.05 [‡]	10.07±0.08	10.26±0.09	1.21±0.05
H ₂ O content	3.35±0.11	2.16±0.13 [‡]	2.88±0.19	3.44±0.15	3.34±0.10
Soleus					
Wet mass	1.14±0.12	0.84±0.04*	0.85±0.01*	0.98±0.05	1.10±0.07
Dry mass	0.32±0.03	0.24±0.01*	0.23±0.004*	0.27±0.01	0.29±0.01
H ₂ O content	0.82±0.08	0.60±0.03*	0.61±0.01*	0.71±0.04	0.81±0.05
EDL					
Wet mass	0.37±0.01	0.35±0.01	0.35±0.01	0.41±0.01	0.40±0.03
Dry mass	0.11±0.004	0.10±0.004	0.10±0.004	0.11±0.01	0.11±0.01
H ₂ O content	0.27±0.01	0.24±0.01	0.25±0.01	0.29±0.004	0.28±0.01
Heart					
Wet mass	3.48±0.09	4.60±0.09 [‡]	5.34±0.22 [‡]	3.50±0.09	3.43±0.12
Dry mass	0.89±0.05	1.18±0.02 [‡]	1.30±0.05 [‡]	0.85±0.03	0.82±0.03
H ₂ O content	2.59±0.08	3.42±0.08 [‡]	4.04±0.17 [‡]	2.65±0.07	2.61±0.10

Wet mass and dry mass are expressed as mg/g BW and H₂O content as µl/g BW. Values are the mean ± S.E.M. (n=8 for all groups). Significance among groups was determined by ANOVA followed by Student–Newman–Keuls test. *P<0.01 vs control. [†]P<0.05 vs control. [‡]P<0.001 vs control.

In addition, other differences between these studies may have accounted for some of the variation in oxygen consumption, such as gender (male vs female), strain of rats (Sprague–Dawley vs Wistar), the route of ligand administration (gavage vs i.p. injection) and diet (cholesterol-fed rats vs regular-fed rats). An important and original finding of the present investigation was that the GC-1-induced increase in VO₂ was not accompanied by an increase in food intake (Fig. 1C) but led to a 20% reduction in body fat content (Fig. 3A–C) without substantially affecting lean or skeletal muscle mass (Table 2).

It is well known that thyroid hormone promotes lipolysis (Wahrenberg *et al.* 1994). In the present study, DEXA analysis showed that animals treated with 10×T3 and 20×T3 presented with a decrease in fat mass of approximately 74 and 91%, while control animals gained fat mass (Fig. 3A). This finding is in agreement with the data from carcass analysis of body composition. The treatments with 10×T3 and 20×T3 reduced the fat content of the animals by 14 and 74% respectively (Fig. 3B). It is noteworthy that GC-1 also decreased the fat mass. The animals treated with 10×GC-1 gained significantly less fat mass than did controls, and rats treated with 20×GC-1 lost approximately 20% of their fat mass (Fig. 3A). The carcass analysis showed that 10×GC-1 and 20×GC-1 reduced the fat content by 15 and 23% respectively, when compared with control animals (Fig. 3B), an effect that was less pronounced than that of T3. This could be explained by a lower uptake of GC-1 by the adipose tissue when compared with the uptake of T3 (Trost *et al.* 2000). In view of the recognised stimulatory effect of thyroid hormone on lipolysis (Moller *et al.* 1996), it is predictable that the

reducing effect of GC-1 on fat mass shown in the present study was a consequence of an increase in lipolysis and not of a reduction in lipogenesis. Indeed, Ribeiro *et al.* (2001) showed that equimolar doses of T3 or GC-1 equally increased the mRNA expression of malic enzyme, an important NADPH-generating lipogenic enzyme, in the livers of mice, suggesting that GC-1, like T3, increases lipogenesis.

It is known that thyroid status strongly influences the contractile function and the mass of the skeletal muscle. However, by DEXA, we could not detect alterations in lean mass induced by T3 or GC-1. These results could be explained by a limited sensitivity of this method of body composition analysis in small animals. In addition, it is important to consider that lean mass by DEXA is determined by the content of minerals that are not from bone, proteins, water and glycogen. As a consequence, changes in these body components, or even the absence of alterations, may mask small or moderate changes in the skeletal muscle mass. In fact, in the present study, the effects of T3 excess on the skeletal muscle were compatible with those described in the literature (White *et al.* 2001). We showed that the quadriceps, gastrocnemius and soleus of animals treated with T3 present lower mass than do those of the control group (Table 2). The EDL mass however was not affected by T3 excess. This corroborates a series of studies that showed that this muscle is weakly responsive to the thyroid status in relation to protein metabolism (Angeras & Hasselgren 1985), muscular function (Nicol & Bruce 1981), the expression of genes related to their function (dos Santos *et al.* 2001) and histological modifications (Devor & White 1995, dos Santos *et al.* 2001). In the present study, we showed that the mass of all skeletal muscles,

except for the quadriceps, was not decreased by GC-1 (Table 2). It is interesting that GC-24, a TR β -selective compound similar to GC-1, mostly spares skeletal muscle from changes in fibre type and tropism induced by T3 (Miyabara *et al.* 2005). The predominance of TR α over TR β (White *et al.* 2001) and/or the poorer penetration of GC-1 in the skeletal muscle (Trost *et al.* 2000), compared with T3, may explain in part the minimal effects of these TR β -selective compounds on this tissue.

In addition to evaluating the effect of T3 or GC-1 on skeletal muscle, we evaluated the effect of these agents on cardiac muscle mass. This analysis was done to obtain positive and negative controls for the treatment with T3 or GC-1 respectively. It is well known that an excess in thyroid hormone causes cardiac hypertrophy (Danzi & Klein 2004) and that GC-1 does not modify the cardiac mass and function (Trost *et al.* 2000). As expected, T3, but not GC-1, increased the heart mass (Table 2), which is a good positive control for the ability of the doses of T3 used in the present study to promote thyrotoxicosis.

We also found that body weight was barely affected by T3 treatment and, different from what has been shown in primates (Grover *et al.* 2004), body weight was not affected by GC-1. T3-treated animals presented a body weight that was lower than that of controls only from day 6 to day 9 and from day 24 to day 27 of treatment (Fig. 1A and B). The absence or minimal effects of T3 on body weight of rats were noticed previously (Yu *et al.* 1998, Soukup *et al.* 2001). In the present study, it can be explained by the increase in food intake induced by T3 (Fig. 1C). On the other hand, GC-1 had no effect on energy ingestion, despite an increase in oxygen consumption at the same magnitude as that with T3 (Fig. 2). The increase in food intake is characteristic of hyperthyroidism. It is believed that one of the mechanisms by which T3 increases food intake involves the progressive depletion of fat stocks, a signal known to regulate the ingestion of energy (Iossa *et al.* 2001). However, T3 was recently shown also to stimulate food intake directly, through the hypothalamic ventromedial nucleus and independently of modifications in energy consumption (Kong *et al.* 2004). Considering the selectivity of GC-1 for TR β and that this T3 analogue increased oxygen consumption, reduced the body fat content, but did not affect food intake, our data suggest that the direct effect of T3 on energy ingestion is essential and likely to be mediated by TR α in rats. Another point to be considered is the lack of effect of GC-1 on body weight (Fig. 1B), which is unusual with regard to energy balance since it increased VO₂ without increasing energy ingestion. The unchanged body weight is possibly explained by the fact that GC-1 significantly reduced body fat content and barely affected muscle mass.

As pointed out before, the TR β selectivity of GC-1 may partially explain some effects of GC-1 observed in the present investigation. If this assumption is true, we could consider that TR β mediates T3 actions that decrease fat mass and increase VO₂, whereas TR α mediates T3 actions that decrease muscle mass and increase food intake. However, GC-1 presents other properties that must be considered and that may contribute to its selective effects. The selective tissue uptake of GC-1 is an

important point, as mentioned before; there is a lower uptake of GC-1 by the skeletal muscle and adipose tissue than of T3 (Trost *et al.* 2000). In addition, a recent *in vitro* study showed that GC-1 behaves largely as a T3 agonist but recruits coactivators or corepressors on some thyroid hormone response elements differently and does this independently of the TR isoform (Gloss *et al.* 2005). The functional consequence of these observations would be a differential regulation of some genes by GC-1 vs T3 that is independent of the GC-1 selectivity for TR β . It seems therefore that the mechanisms underlying the differential effects of GC-1 vs T3 on the biological systems, including body composition, depend on a series of different properties of these ligands, besides the TR β selectivity of GC-1, which remains to be further investigated.

In conclusion, this is the first study showing that treatment with GC-1 decreases the body content of adipose tissue while it does not increase food intake and barely affects the mass of skeletal muscle. The reduction of body fat content added to other GC-1 effects previously described, such as reduction of serum levels of cholesterol, triglycerides and TSH (Trost *et al.* 2000, Freitas *et al.* 2003, Grover *et al.* 2004), and the lack of deleterious effects of GC-1 on the heart structure and function (Trost *et al.* 2000), skeletal muscle mass and bone mass (Freitas *et al.* 2003) suggest that GC-1 has the potential to be used as a therapeutic agent for a variety of disorders, such as obesity, hyperlipidaemia and hypercholesterolaemia.

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